Fundamentals
of
High Resolution
Pulse and Fourier Transform
NMR Spectroscopy

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Part I
Lecture Notes

by

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1. The Nuclear Zeeman Effect.

1.1. A magnetic field breaks the degeneracy of nuclear spin states.

When a nuclear spin is placed in a magnetic field the degeneracy associated with the *z* component of angular momentum is lifted. For a nucleus of angular momentum, $I = 1/2$, two energy levels result as shown in Figure 1.

The angular frequency, ω_0 , is referred to as the resonance or Larmor frequency of the nuclear spin. Each type of nucleus has a characteristic gyromagnetic factor, γ , which, together with the strength of the external magnetic field, $\mathbf{B}_0$, determines the Larmor frequency. If $\mathbf{B}_0 = 7.05$ Tesla , for example, then, for protons, $\nu_0 = \omega_0/2\pi = 300$ MHz , and for ^{13}C , $\nu_0 = 75$ MHz . Of course, these are nominal values which are affected by chemical shifts, etc. Also, γ can be negative, as in ^{15}N for example, causing the spins to precess in the opposite sense.

1.2. The differences in level populations are very small.

Problem 1: A certain sample has 1 million chloroform molecules. At 300 K using Boltzmann statistics, how many fewer protons will have $m = -1/2$ than have $m = 1/2$ on a 7.05 Tesla spectrometer.

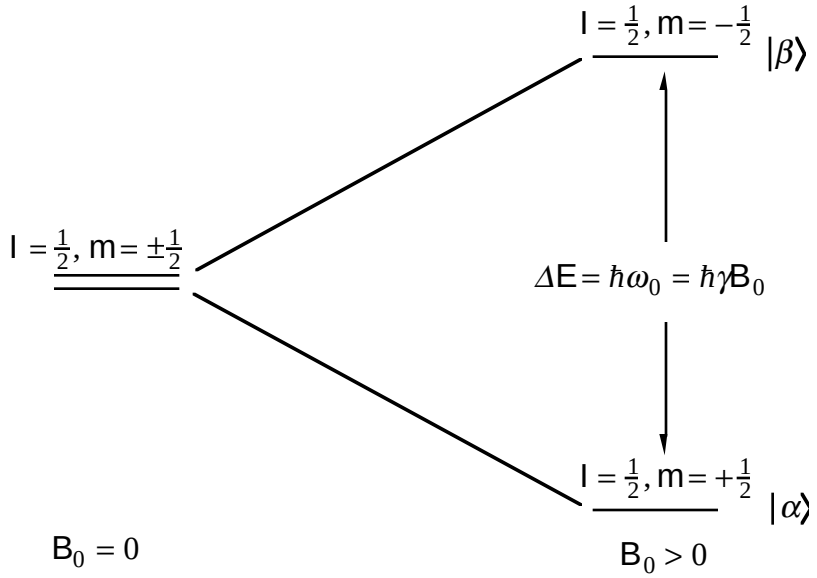


Figure 1

Answer:

$$kT = 4.14 \times 10^{-14}\,\text{erg}$$

$$\Delta E = 1.99 \times 10^{-18}\,\text{erg}$$

$$\frac{\Delta E}{kT} = 4.8 \times 10^{-5}$$

$$\exp\left(-\frac{\Delta E}{kT}\right) = 0.999952 = \left(\frac{5 \times 10^5 - \delta/2}{5 \times 10^5 + \delta/2}\right)$$

$$\delta = 24$$

2. Net Magnetization and Nuclear Precession.

2.1. Classical Equation of Motion of a Magnetic Dipole.

The classical equation of motion for a magnetic moment, **μ**, in an external field, **B**, is given by:

$$\frac{\mathrm{d}\mathbf{p}}{\mathrm{d}t} = \mu \times \mathbf{B} \tag{1}$$

where **p** is the angular momentum. For nuclear spins, **p** and **μ** are related by the gyromagnetic ratio, γ, according to:

$$\mu = \gamma \mathbf{p} \tag{2}$$

Eliminating **p** between Equation (1) and Equation (2) one obtains:

$$\frac{\mathrm{d}\mu}{\mathrm{d}t} = \gamma(\mu \times \mathbf{B}) \tag{3}$$

Note that the time rate of change of **μ** is always perpendicular to **μ** and **B**. See Figure 2.

2.2. Magnetization of an Ensemble of Spins.

Now suppose one has an ensemble of moments, μ_i, each making an angle, θ, with the external field (the external field direction will be taken to be the *z* direction), but randomly distributed with respect to their components in the *xy* plane. When nuclear spins are treated quantum mechanically they distribute in just this way if they are placed in a magnetic field for a sufficient length of time. The dipoles will have identical magnitudes but a

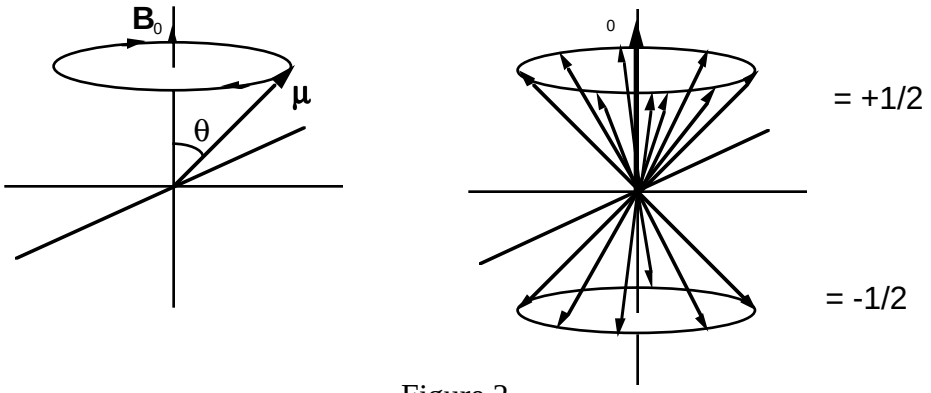


Figure 2

few more will have a positive *z* component rather than a negative one. This corresponds to the excess of nuclear spins having $m = 1/2$ over those having $m = -1/2$. (See Figure 2.) Summing over all the μ_i, one has:

$$\sum_i \mu_i = \mathbf{M}_0 \tag{4}$$

$\mathbf{M}_0$ is usually referred to as the thermal equilibrium magnetization.

2.3. Nuclear Precession.

Rather than the thermal equilibrium distribution described above, suppose that the μ_i are arbitrarily distributed such that

$$\sum_i \mu_i = \mathbf{M} \tag{5}$$

then it is easy to show that

$$\frac{\mathrm{d}\mathbf{M}}{\mathrm{d}t} = \gamma(\mathbf{M} \times \mathbf{B}) \tag{6}$$

Problem 2: Derive Equation (6).

Problem 3: Write the above vector equation in component form and show that

$$\begin{aligned} \frac{\mathrm{d}M_x}{\mathrm{d}t} &= \gamma\left(M_y B_z - M_z B_y\right) \\ \frac{\mathrm{d}M_y}{\mathrm{d}t} &= -\gamma\left(M_x B_z - M_z B_x\right) \\ \frac{\mathrm{d}M_z}{\mathrm{d}t} &= \gamma\left(M_x B_y - M_y B_x\right) \end{aligned} \tag{7}$$

Figure 3 illustrates the evolution of **M** according to Equation (6) or Equation (7) when **B** is in the *z* direction and **M** is in the *yz* plane at time, *t*.

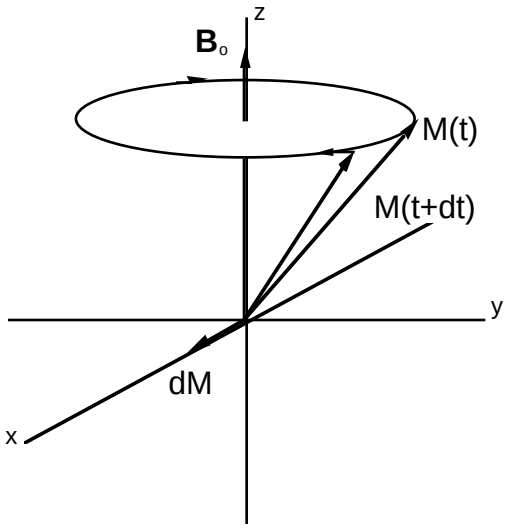


Figure 3

3. Detection of Precessing Magnetization

In the case where $\mathbf{B} = B_0\mathbf{k} = B_z\mathbf{k}$ and $\mathbf{M}(t=0) = M_0\mathbf{i} = M_x\mathbf{i}$ the equation of motion for **M** reduces to

$$\frac{\mathrm{d}\mathbf{M}}{\mathrm{d}t} = \gamma\left(M_y B_z\mathbf{i} - M_x B_z\mathbf{j}\right) \tag{8}$$

Problem 4: Satisfy yourself that the above equation is correct and prove that a solution of this equation is

$$\mathbf{M}(t) = M_0\cos(\omega_0 t)\mathbf{i} - M_0\sin(\omega_0 t)\mathbf{j} \tag{9}$$

where $\omega_0 = \gamma B_0$.

Now, if a coil is wrapped around the sample as in Figure 4, an oscillating voltage will be induced in the coil by the oscillating magnetization in the sample. This oscillatory behavior occurs only when **M** has *x* or *y* components. Recall that this is not the case at ther-

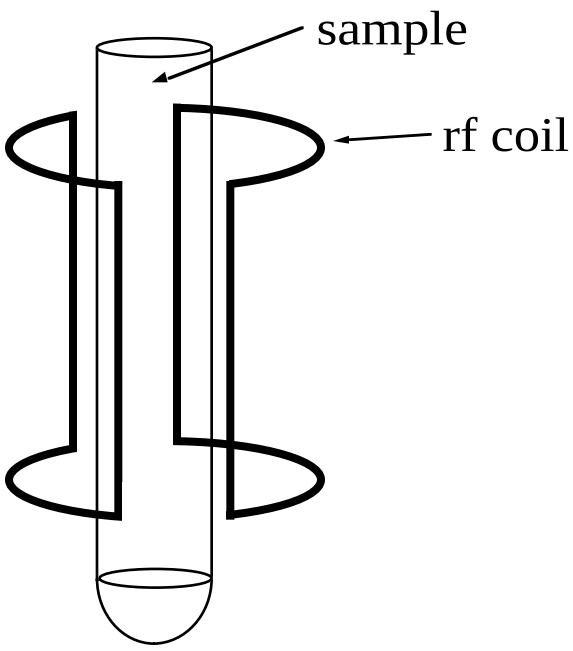


Figure 4

mal equilibrium. This voltage is generally of the order of microvolts but can be amplified and recorded. This is the NMR signal.

4. The Rotating Frame.

Consider a coordinate system rotating about the z-axis of a laboratory fixed frame at an angular frequency, ω. The rotating (primed) coordinate system is then related to the laboratory fixed system by the transformation:

$$\begin{aligned} x' &= x\cos(\omega t) + y\sin(\omega t) \\ y' &= -x\sin(\omega t) + y\cos(\omega t) \\ z' &= z \end{aligned} \tag{10}$$

Problem 5: Show that under this transformation the equation of motion for the bulk magnetization becomes

$$\frac{\mathrm{d}\mathbf{M}'}{\mathrm{d}t} = \gamma\left(\mathbf{M}' \times \mathbf{B}_{eff}\right) \tag{11}$$

where

$$\mathbf{B}_{eff} = \mathbf{B}' - \left(\frac{\omega}{\gamma}\right)\mathbf{k}' \tag{12}$$

Thus, in the rotating frame, the equation has the same form as in the laboratory frame, except that the *z* component of the external field is reduced by a factor, ω/γ. (This factor is often called the fictitious field.) This means that the solutions will have the same form as in the laboratory frame except that the precession frequency will be the difference between the Larmor frequency and the rotating frame frequency. In what follows the primes will not be carried, but it should be assumed that all equations are in a rotating frame. The rotating frame used should be evident from the context. Figure 5 illustrates these results for the case where **B** is along the *z*-axis**;** note that, if $\omega_0 = \omega$, then $\mathbf{B}_{eff} = 0$ and **M** is stationary in the rotating frame. This condition is termed resonance. Without dwelling on the details of the electronics, one can say that an NMR spectrometer has the capacity to transform the signals from the probe (laboratory frame) to equivalent signals in a frame rotating at ω_{rf}.

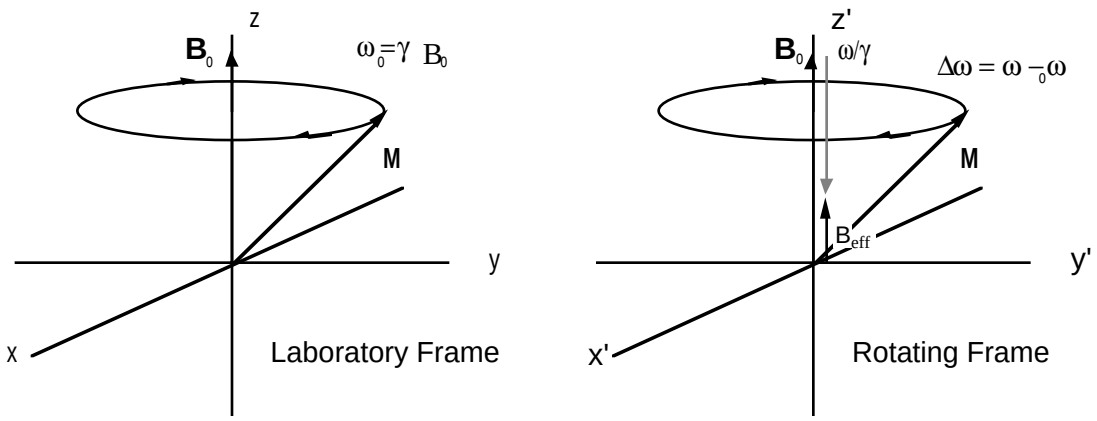

z
B0
ω0=γ B0
M
y
x
Laboratory Frame
z'
B0
ω/γ
Δω = ω −0ω
M
Beff
y'
x'
Rotating Frame

Figure 5

5. The Effects of Radio Frequency Fields—Pulses.

5.1. On Resonance Pulses.

Recall that when a sample is placed in a magnetic field, the magnetization goes (after some time) to thermal equilibrium with no transverse components and, consequently, no NMR signal in the coil. How can one perturb the magnetization away from thermal equilibrium so as to produce transverse components which can be detected?

Using the same coil shown in Figure 5, one can apply an oscillating voltage to the coil so as to create an oscillating magnetic field in the sample. This oscillating field can be decomposed into two counter rotating fields. One or the other of these fields will always be rotating in the same sense as the precession of the nuclear spins. The form of this rotating field is

$$\mathbf{B}_1 = B_1\left[\cos\left(\omega_{rf}t\right)\mathbf{i} + \sin\left(\omega_{rf}t\right)\mathbf{j}\right] \tag{13}$$

Problem 6: Show that the sum of two counter rotating fields is an oscillating field.

If all three of the frequencies: the oscillating field, ω_{rf} ; the rotating frame, ω; and the Larmor frequency, ω_0 ; are made equal, then the equation of motion has the form

$$\frac{\mathrm{d}\mathbf{M}}{\mathrm{d}t} = \gamma\mathbf{M}\times\mathbf{B}_1 \tag{14}$$

Problem 7: Convince yourself that Equation (14) follows from those given previously.

This equation says that the magnetization will precess about the direction of the rf field at a rate $\omega_1 = \gamma B_1$. A typical value of ν_1 might be 25 KHz. This means that a complete rotation of the magnetization through 360 degrees would take 40 μs. Suppose, however, that we turn on the rf transmitter for only 10 μs, delivering a pulse of energy to the sample which precesses the magnetization through 90 degrees and leaves it in the xy plane. As shown in Figure 6, by controlling the duration and phase (the phase controls the orientation of $\mathbf{B}_1$ with respect to the rotating frame axes) of the rf pulse the magnetization can be placed anywhere one desires.

Problem 8: Make a figure similar to Figure 6 showing how the magnetization could be placed along the $+x$ or $-y$-axes.

5.2. Off Resonance Pulses.

If $\omega_0 \neq \omega$, then $\mathbf{B}_{eff}$ has a component in the z direction because the fictitious field from the rotating frame transformation does not perfectly cancel $\mathbf{B}_0$. As shown in Figure 7, $\mathbf{B}_{eff}$ is the sum of $\mathbf{B}_1$ and this "off resonance" field. The magnetization then precesses about $\mathbf{B}_{eff}$ and not about $\mathbf{B}_1$. The magnetization traverses the surface of a cone with $\mathbf{B}_{eff}$ as its axis.

Problem 9: If $\mathbf{B}_{eff}$ differs appreciably from $\mathbf{B}_1$, it is not possible to produce an accurate 180 degree pulse. Explain why and calculate the magnitude of the error for the best approximation to a 180 degree pulse.

Problem 10: If $\omega_0 \neq \omega_{rf}$, will the time necessary to produce, as nearly as possible, a 180 degree pulse be longer or shorter than when

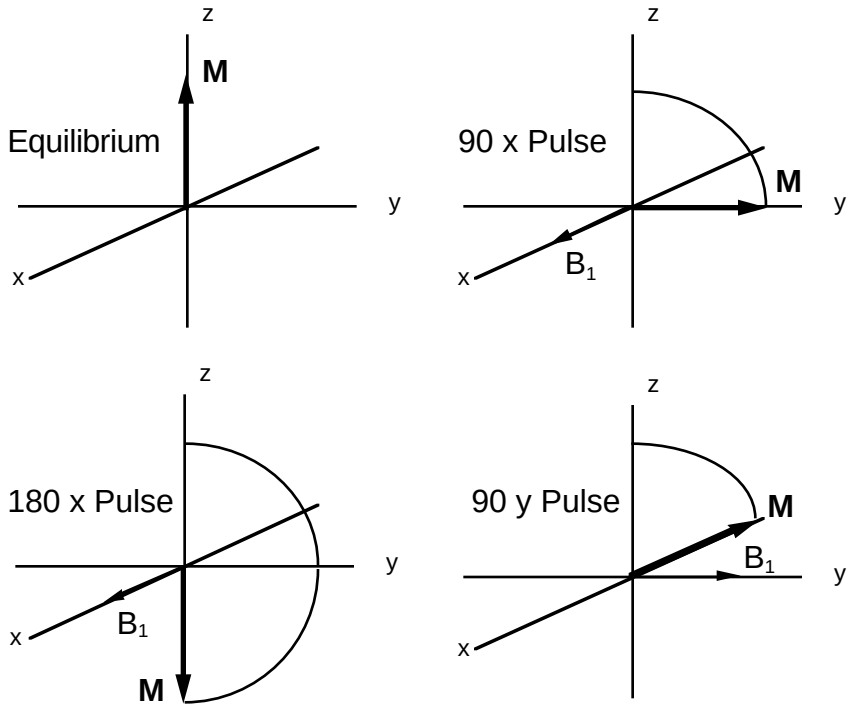


Figure 6

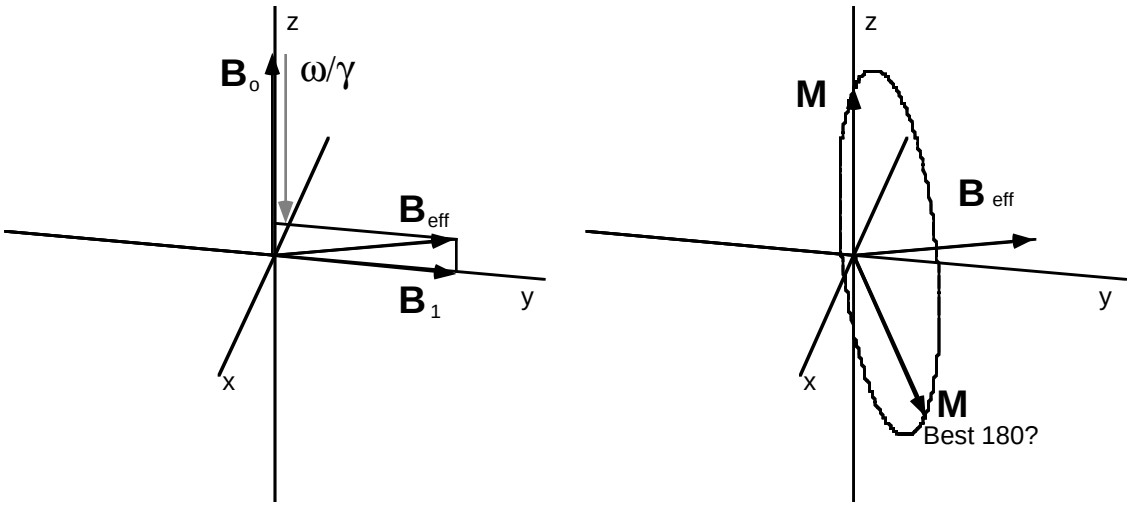


Figure 7

$\omega_0 = \omega_{rf}$? Why? Will the phase of the magnetization for a 90 degree pulse be the same as for an on resonance pulse? In terms of these concepts explain why carbon-13 isn't perturbed by a pulse applied to protons.

6. Relaxation—Bloch's Equation.

Thus far our equations provide no means for the magnetization, once perturbed, to return to thermal equilibrium. Since, experimentally, the magnetization is observed to return to thermal equilibrium, one must introduce dissipative terms which will allow this to happen. Addition of these terms to the previous equation of motion yields the equation of motion referred to as Bloch's equation.

$$\begin{aligned}\frac{\mathrm{d}M_x}{\mathrm{d}t} &= \gamma\left(\mathbf{M}\times\mathbf{B}\right)_x - \frac{M_x}{T_2}\\ \frac{\mathrm{d}M_y}{\mathrm{d}t} &= \gamma\left(\mathbf{M}\times\mathbf{B}\right)_y - \frac{M_y}{T_2}\\ \frac{\mathrm{d}M_z}{\mathrm{d}t} &= \gamma\left(\mathbf{M}\times\mathbf{B}\right)_z - \frac{M_z - M_0}{T_1}\end{aligned} \tag{15}$$

In the absence of all external fields but $\mathbf{B}_o$ and with $\omega = \omega_o$, each component of $\mathbf{M}$ decays independently and exponentially to thermal equilibrium. This process is called relaxation.

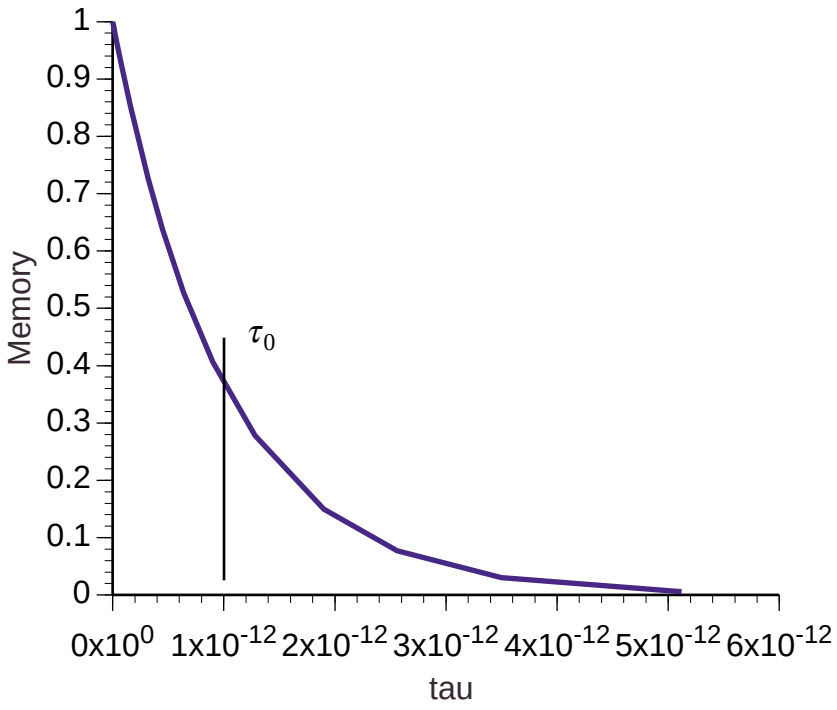
Problem 11: Show that, under the above stated restrictions, each of the above equations has a solution of the form

$$M = A^{-\alpha t} + C \tag{16}$$

What are A, C, and α in each case?

The decay of M_x and M_y is called transverse or spin-spin or T_2 relaxation, and that of M_z is called longitudinal, spin-lattice, or T_1 relaxation.

Problem 12: If one now assumes that $\omega \neq \omega_o$, what is the form of Bloch's equation? This is the equation of motion for a Free Induction Decay (FID). Recall that Equation (8) deals with this case in the absence of relaxation.

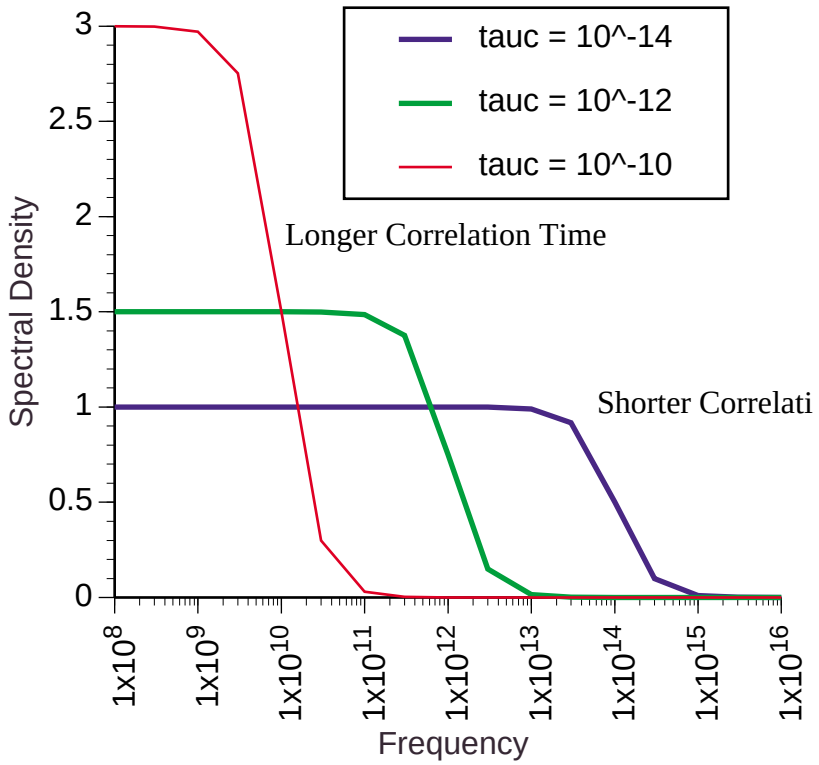


6.1. Correlation Functions and Spectral Densities.

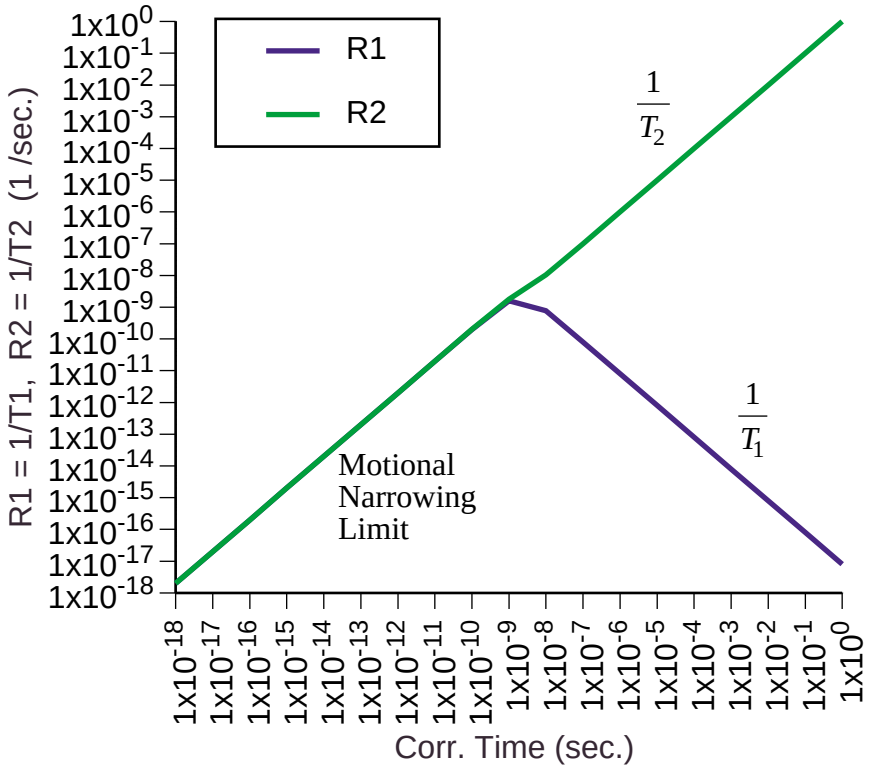
The rotational correlation time is a measure of how fast a molecule is tumbling in a liquid. The spin-lattice and spin-spin relaxation times are related to the rotational correlation time by

$$\frac{1}{T_1} = \gamma^2\left(\overline{B_x^2} + \overline{B_y^2}\right)\frac{\tau_0}{1+\omega_0^2\tau_0^2}$$
$$\frac{1}{T_2} = \gamma^2\left[\overline{B_z^2}\tau_0 + \frac{1}{2}\left(\overline{B_x^2} + \overline{B_y^2}\right)\frac{\tau_0}{1+\omega_0^2\tau_0^2}\right] \qquad (17)$$

where $\overline{B_q^2}, q = x, y, z$ is the average of the square of the strength of a random field acting on a nucleus, ω_0 is the Larmor frequency, and τ_0 is the correlation time of the molecular tumbling. This correlation time gives a measure of the time required for the molecule to lose its memory of rotational position as shown in Figure 8. This is called a correlation function. The Fourier transform of a correlation function is called a spectral density func-



tion. The amplitude of a spectral density function at a certain frequency controls the relaxation rate. Figure 9 shows examples of spectral density functions for several different correlation times. The area under each spectral density curve is the same. Figure 10 shows relaxation times as a function of correlation time. Note how the spin-lattice rate reaches a maximum then declines while the spin-spin rate continues to increase. Thus, small molecules in nonviscous media show long relaxation times and narrow lines because they are tumbling rapidly. This is called motional narrowing or extreme narrowing. Note also that $T_1 = T_2$ in this region. By contrast, large molecules, viscous media, or solids are characterized by long spin-lattice relaxation times, but wide lines because the spin-spin relaxation times are growing ever shorter. Actually, the spin-spin relaxation rate reaches a limit when all molecular tumbling stops, as in many crystalline solids at low temperature. This effect is not shown in the figure. The maximum of the spin-lattice curve occurs when $\omega_0 \tau_0 = 1$. This means that when the field strength of a spectrometer is increased, the correlation time



corresponding to the maximum will decrease, but the motional narrowing limit will remain the same.

6.2. Relaxation Mechanisms.

Any process that causes the magnetic field seen by the nucleus to fluctuate randomly can cause relaxation.

The following are the most important relaxation mechanisms for high resolution NMR of liquids. They are arranged roughly in order of decreasing importance for organic molecules.

6.2.1. Dipole-Dipole Interactions.

Every other magnetic nucleus in a sample will create a magnetic field at a nucleus of interest. The nuclei can be in the same or different molecules. The strength of the interaction is inversely proportional to the sixth power of the distance between the nuclei; thus,

the nuclei must be very near each other (within a few Angstroms) for a significant interaction to occur. The modulation is caused by rotation and translation of the nuclei.

This interaction can be exploited to measure distances between nuclei.

6.2.2. Chemical Shift Anisotropy.

We measure chemical shifts as the position of lines in a spectrum relative to some standard like TMS. Actually the chemical shift depends on the orientation of a molecule with respect to the external field. In liquids the molecules tumble rapidly with no preferred orientation, so that the chemical shift we measure is an average of that for all possible orientations of the molecule. However, modulation of the chemical shift as the molecule tumbles produces relaxation. The strength of this interaction is proportional to the square of the external field. For a 300 MHz or lower spectrometer this effect is usually negligible, but at 500 MHz and above it can be significant.

6.2.3. Paramagnetic Interactions.

Since the electron has a spin and its magnetic moment is about 2000 times greater than that of nuclei, dipole-dipole interactions of nuclei with electrons can be a dominant relaxation mechanism. Fortunately, most electrons come in pairs of opposite spin and cancel each other out. Molecules or ions with unpaired electrons are called paramagnetic. Free radicals, and metal ions of iron or chromium are examples. If such species are present in a NMR sample, even in very low concentration, T_2 relaxation may be so rapid that nuclear spin signals are very broad and cannot be resolved from each other. Molecular oxygen is mildly paramagnetic, so dissolved oxygen does contribute to relaxation.

6.2.4. Spin-Rotation Interactions.

For small molecules rotating rapidly and undergoing collisions with other molecules, interaction of the magnetic field produced as the molecule rotates with the nuclear magnetic moment can produce relaxation. For most organic molecules this effect is negligible. Dissolved CO_2 would be an example where it would be non-negligible. This mechanism becomes more important as the temperature is increased in contrast to other mechanisms which vary inversely with temperature.

6.2.5. Quadrupolar Interactions.

If a nucleus has a quadrupole moment, deuterium for example, and is in an unsymmetrical electronic environment, this can produce a fluctuating field that causes relaxation. Since carbon-13 and protium have no quadrupole moment, this mechanism is often unimportant for structural organic chemistry.

6.2.6. Scalar Interactions.

Just as for dipole-dipole and quadrupolar interactions, scalar interactions or J-couplings depend on molecular orientation and the values we see in liquid spectra are averages over all orientations. Likewise, the modulations involved can produce relaxation. However, this effect is usually negligible in organic molecules.

7. The CW NMR Experiment.

Now suppose one turns on a $\mathbf{B}_1$ field along the *x* direction of the rotating frame. The equation of motion becomes

$$\begin{aligned}\frac{\mathrm{d}M_x}{\mathrm{d}t} &= \gamma M_y \Delta B - \frac{M_x}{T_2} \\ \frac{\mathrm{d}M_y}{\mathrm{d}t} &= -\gamma M_x \Delta B + \gamma M_z B_1 - \frac{M_y}{T_2} \\ \frac{\mathrm{d}M_z}{\mathrm{d}t} &= -\gamma M_y B_1 - \frac{M_z - M_o}{T_1}\end{aligned} \tag{18}$$

where

$$\Delta B = \frac{\omega - \omega_o}{\gamma}$$

Problem 13: If $\mathbf{B}_1$ is small show that the steady state solution of these equations is

$$\begin{aligned}M_x &= \chi_o \omega_o T_2 \frac{(\omega - \omega_o)T_2}{1 + (\omega - \omega_o)^2 T_2^{\,2}} B_1 \\ M_y &= \chi_o \omega_o T_2 \frac{1}{1 + (\omega - \omega_o)^2 T_2^{\,2}} B_1 \\ M_z &= M_o\end{aligned} \tag{19}$$

where

$$\chi_o = \frac{M_o}{B_o}$$

Suppose one keeps $\mathbf{B}_o$ and $\mathbf{B}_1$ constant and changes ω slowly. Then the NMR signal has the form

$$\begin{aligned}F_x(\omega) &= A T_2 \frac{(\omega - \omega_o)T_2}{1 + (\omega - \omega_o)^2 T_2^{\,2}} B_1 \\ F_y(\omega) &= A T_2 \frac{1}{1 + (\omega - \omega_o)^2 T_2^{\,2}} B_1\end{aligned} \tag{20}$$

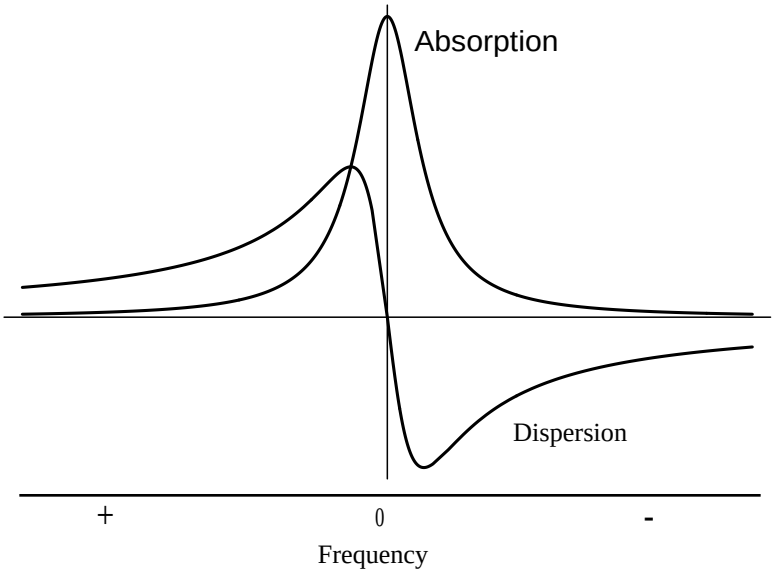


Figure 11

where A represents various constants including the efficiency with which one can detect and amplify the voltage induced in the receiver coil. This equation represents the basic continuous wave (cw) NMR experiment. Figure 11 shows the form of these two signals. F_x represents what is termed the dispersion mode signal, while F_y is the absorption mode. The latter is the signal usually displayed when one records a cw spectrum.

Problem 14: Make a scaled plot similar to Figure 11. What is the effect on the curves of changing B_1 or T_2? What is the full width at half maximum (fwhm) of the absorption curve? How many fwhm units does it take for the absorption mode to fall to one percent of its maximum value? How many for the dispersion signal?

8. The FT NMR Experiment.

If one applies a 90 degree pulse along the *x*-axis, the magnetization is left along the *y*-axis. The magnetization as a function of time, subsequent to the pulse, has the form

$$\begin{aligned} M_x &= M_0 e^{-(t/T_2)} \sin\left[(\omega_0 - \omega)t\right] \\ M_y &= M_0 e^{-(t/T_2)} \cos\left[(\omega_0 - \omega)t\right] \end{aligned} \tag{21}$$

The NMR signal (FID) detected by the spectrometer is, of course, proportional to this magnetization (see Figure 12). It is convenient to define a complex FID as

$$f(t) = M_y + iM_x \tag{22}$$

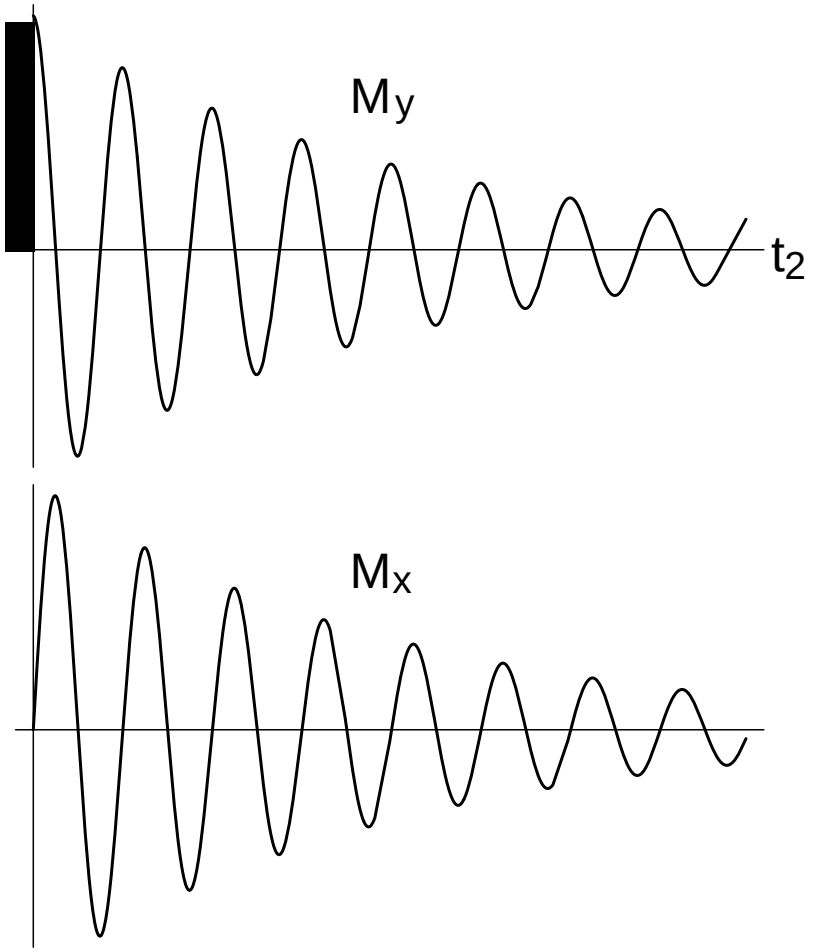


Figure 12

A Fourier transform (FT) is then performed on this FID according to

$$F(\omega) = \int_{-\infty}^{\infty} f(t) e^{-i\omega t} \tag{23}$$

resulting in

$$F(\omega) = \frac{M_0 T_2}{2}\left(\frac{1}{1+(\omega-\omega_0)^2 T_2^2} + i\frac{\omega-\omega_0}{1+(\omega-\omega_0)^2 T_2^2} \right) \tag{24}$$

Comparing Equation (24)with the results of the cw experiment, Equation (20), the real part of $F(\omega)$ corresponds to the absorption mode signal and the imaginary part to the dispersion mode. Figure 12 shows a complex FID and the corresponding FT spectrum is identical to that shown in Figure 11.

> Problem 15: What would be the difference between a FID from a resonance above the rotating frame in frequency as opposed to one whose frequency is less than that of the rotating frame by the same amount?

If one has a spectrum with many lines, a pulse may be applied to simultaneously excite all of the lines and a FID collected containing the spectral information from all the resonances. A Fourier transform can then be performed to produce a spectrum containing all the information that, by cw methods, would require slowly sweeping through each resonance and recording the result.

9. The Anatomy of a Free Induction Decay.

Intelligent operation of an NMR spectrometer requires a clear understanding of the FID and its relationships to the transformed spectrum. Since all processing of the FID is done using a digital computer, it is also important to understand the consequences of converting the FID to digital form.

9.1. FID of a Single Resonance.

Figure 12 shows the complex FID of a single resonance such as might be obtained from a sample of chloroform and Figure 11 shows the Lorentzian line which would be obtained from Fourier transforming this FID. They have the following properties:

a. The initial amplitude of the FID is proportional to the height or the integrated intensity of the line.

b. The time constant, T_2^* of the exponential decay of the FID is related to the full width at half maximum, L, of the absorption mode signal by

$$L = \frac{1}{\pi T_2^*} \tag{25}$$

Thus, FID's with short time constants give wide lines and FID's with long time constants give narrow lines.

c. The frequency of the oscillation is $\Delta\omega$, the frequency difference between the transmitter and the line.

d. The phase of M_x indicates the sign of the frequency. If counterclockwise rotation (as viewed from the positive z-axis) is taken to be positive, then the FID as shown represents a negative frequency. If the phase of M_x is shifted by 180 degrees, then the frequency would be positive.

9.2. FID of Two or More Lines.

The FID of two or more lines is obtained by summing the FID's corresponding to the individual lines. As shown in Figure 13 (a), two lines give a fairly simple FID. In this case the two lines have frequencies of 20 and 25 Hz. The frequency associated with the interference beats gives the separation of the two lines, 5 Hz in this case. However, as one adds more lines to the FID, it quickly becomes too complicated to analyze visually.

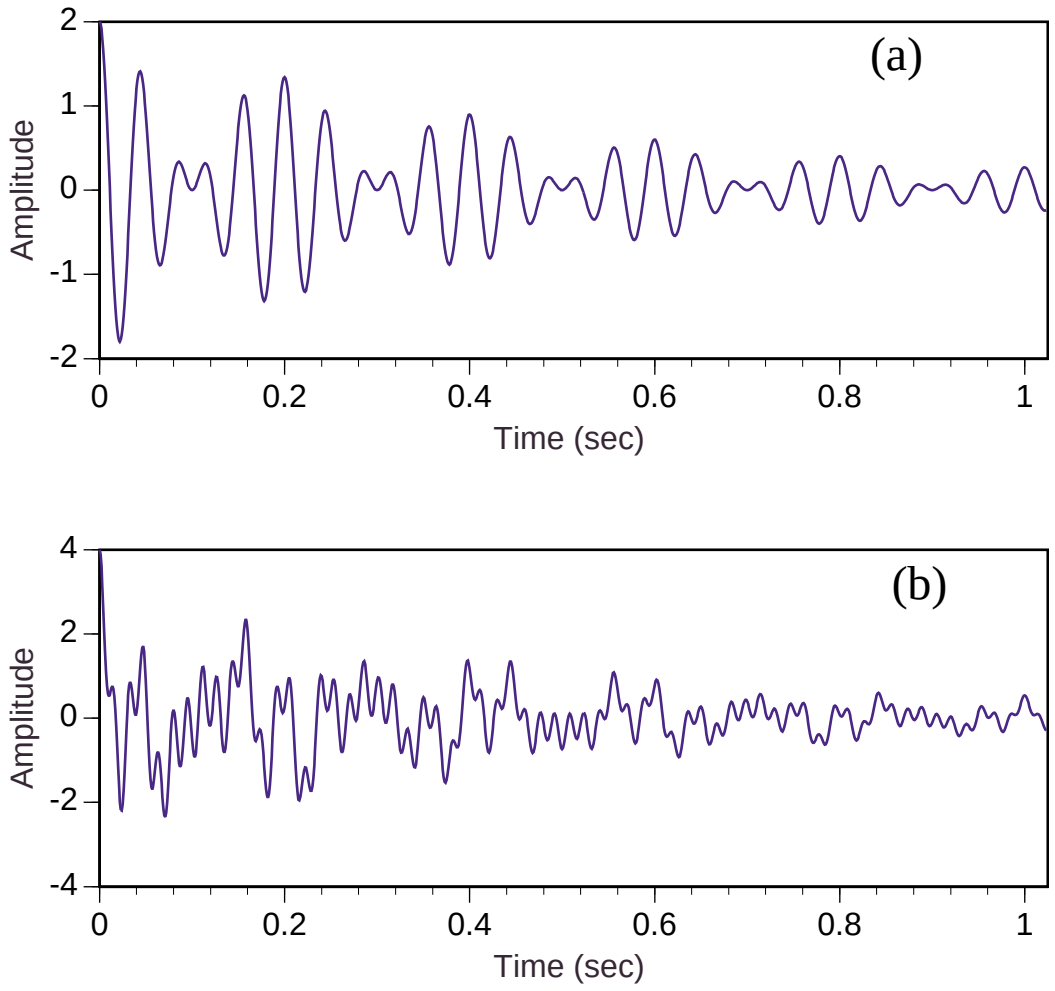


Figure 13 (b) shows a FID with four lines. Lines at 7 and 63 Hz have been added to the FID of (a).

9.3. Digitization of Free Induction Decays.

9.3.1. Digitization in Amplitude.

The FID is sampled by the sample and hold unit (S/H), converted to digital form by the analog to digital converter (ADC), and stored in the computer memory.

The dynamic range of the digitized FID and, consequently, of the spectrum depends on the number of bits in the ADC.

Noise can be introduced due to truncation errors.

See Figure 14 for an illustration of this process.

Problem 16: What is the minimum number of ADC bits required to digitize the ^{1}H FID from tert-butyl alcohol? Assume there is no scalar coupling. How many bits would be required to digitize the ^{1}H FID from a 5 mM solution of sodium dichloroacetate in 90% H_2O/10% D_2O?

9.3.2. Digitization in Time.

The S/H unit is triggered at precisely equal intervals of time by a signal derived from the same master frequency from which all other frequencies in the spectrometer are derived. Both M_x and M_y are sampled simultaneously. Figure 15 shows schematically how the S/H works.

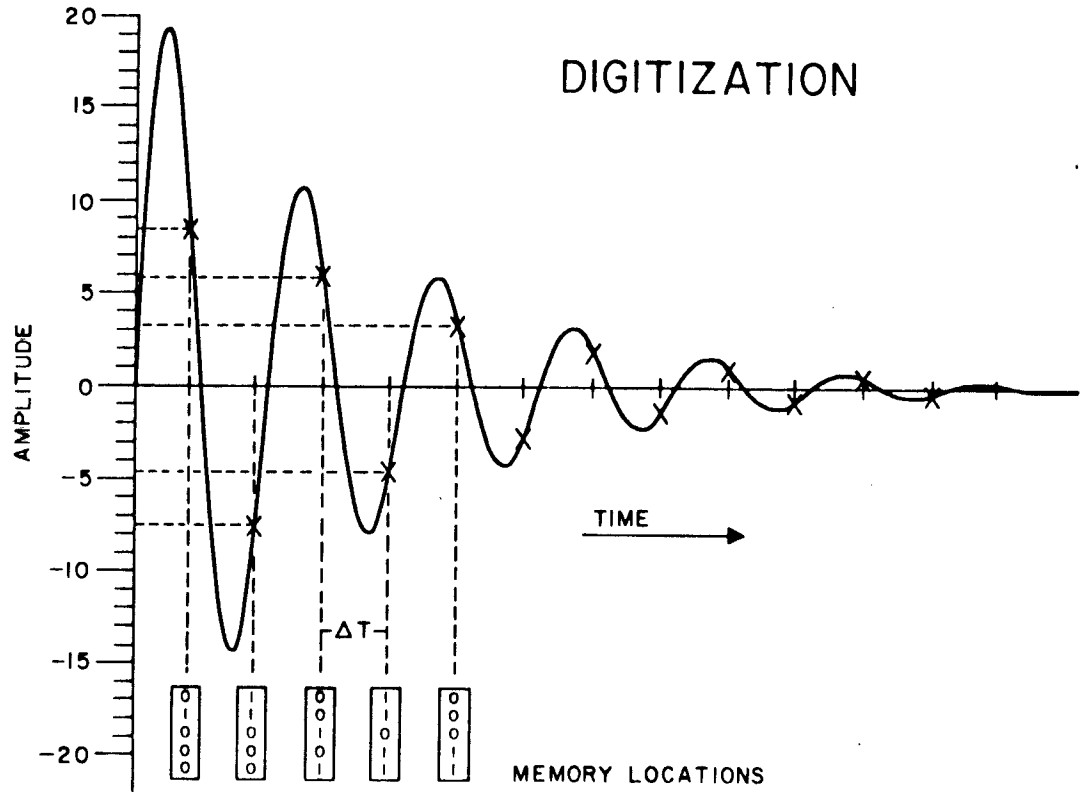


Figure 14

The spectral width, *S*, over which resonances can be accurately sampled is given by

$$S = \frac{N}{A} \tag{26}$$

where *N* is the number of complex samples of the FID (The number of points parameter on most spectrometers is $2N$, counting one for the real and one for the imaginary part of the FID.) and *A* is the total time during which the FID is sampled, the acquisition time. The spectral width encompasses the range $\pm S/2$ from the frequency of the transmitter.

The interval between data points in the FID will be $A/N = 1/S$ and the separation in the spectrum will be $S/N = 1/A$.

Problem 17: Assuming that two adjacent lines are narrower than $1/A$, how close to each other can they be and still be detected as separate lines? Suppose that this separation were due to a scalar coupling; what error limits should you report for the coupling constant?

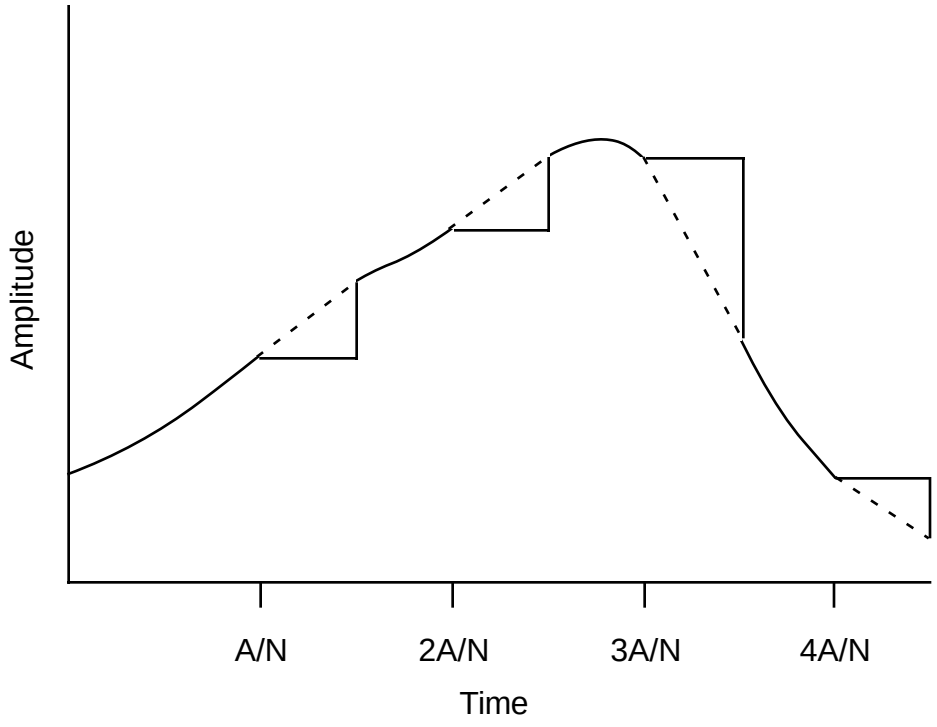


Figure 15

If the computer memory is capable of accommodating only N_{max} complex data points, then the product of *S* and *A* cannot exceed this value.

> Problem 18: Find out what the value of N_{max} is for the spectrometer you are using and compute the best resolution you can obtain using a spectral width sufficient to cover the nominal maximum chemical shift range for ^{1}H. Do the same for ^{13}C. If you have shimmed the magnet to 0.2 Hz line width, what is the maximum spectral width you can use while retaining your hard won resolution?

Resonances which occur outside the spectral width defined by the sampling rate are aliased to a point within the spectral width as shown in Figure 16. This phenomenon is often incorrectly referred to as foldover rather than aliasing.

If only the real or only the imaginary part of the FID is acquired, the ability to discriminate between signals above and below the transmitter in frequency is lost, and each line will appear on both sides of the carrier and equidistant from it. One will be a phantom image and the other the real line. One cannot distinguish between the two without additional information. This phenomenon is correctly termed foldover because it is as though the spectrum were folded about the transmitter frequency and the two halves superimposed.

> Problem 19: If the transmitter is moved a small amount or the spectral width is changed by a small amount, how will images, real lines, and aliased lines respond? How can this information be used to detect the false lines?

9.4. Time Averaging.

Suppose one acquires *N* identical FID's. Each sample of each FID contains a signal component, *S*, and a noise component which will be supposed to be random (Gaussian distributed) with standard deviation σ_S. Then, if one adds the FID's, the signal components will add coherently to give a total signal, S_t, according to

$$S_t = NS \tag{27}$$

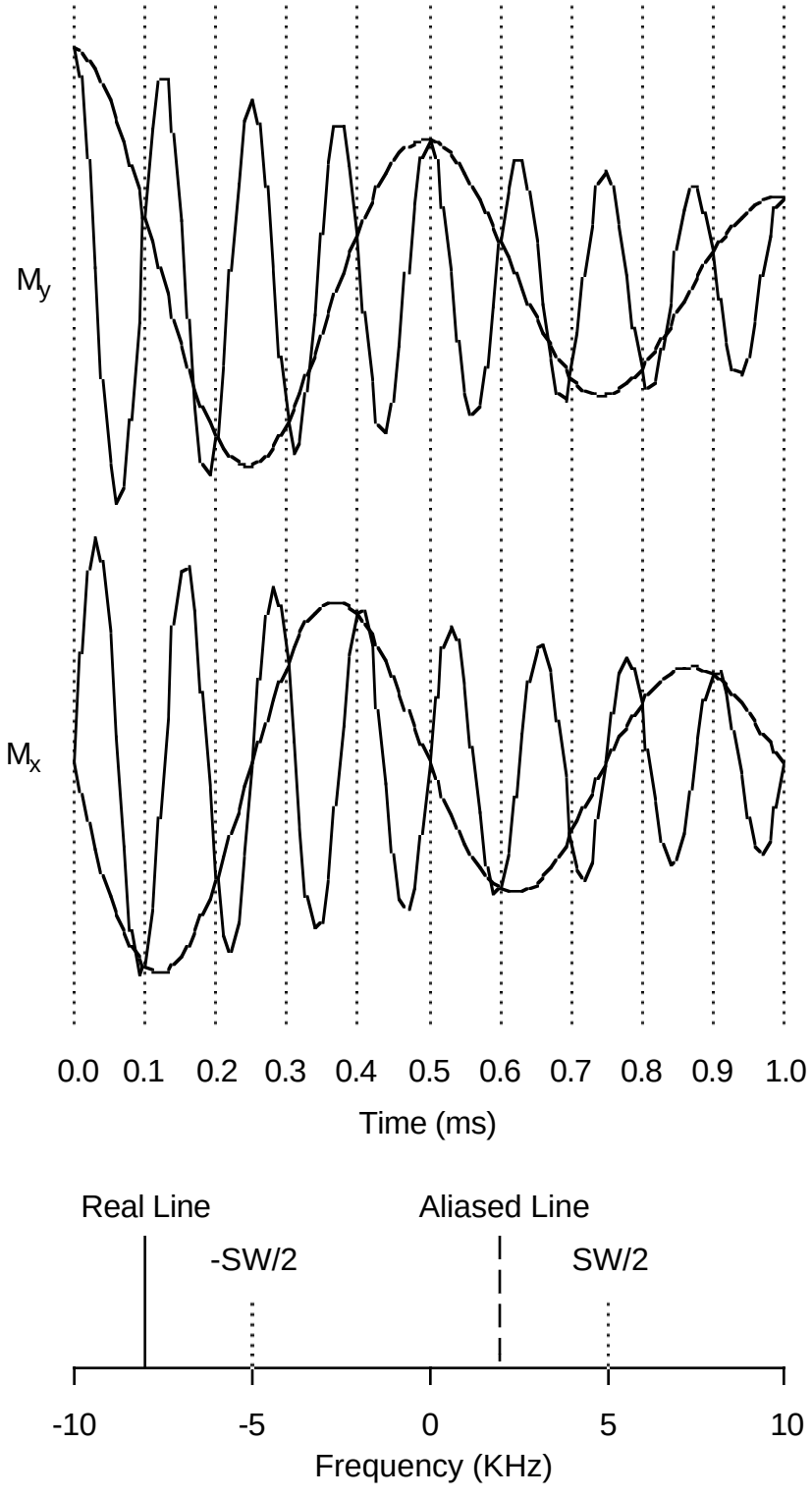

My
Mx
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Time (ms)
Real Line
Aliased Line
-SW/2
SW/2
-10 -5 0 5 10
Frequency (KHz)

Figure 16

But it can be shown that σ_t, the standard deviation of the noise in the time averaged FID, is given by

$$\sigma_t = \sqrt{N}\sigma_S \tag{28}$$

Hence, taking the ratio of the signal to the noise in the summed FID

$$\bar{S}_t = \frac{S_t}{\sigma_t} = \frac{NS}{\sqrt{N}\sigma_S} = \sqrt{N}\frac{S}{\sigma_S} \tag{29}$$

Thus, the signal to noise ratio $\bar{S}_t$ of a time averaged FID increases as the square root of the number of FID's averaged.

> Problem 20: After running a sample all day (say 12 hours) you are beginning to see what you think are signals (signal to noise ratio about 2). How much longer will it take to get a publishable spectrum (signal to noise ratio about 10)?

The fact that one is time averaging digitized FID's also has some consequences of which one needs to take account when planning experiments. Time averaging cannot produce a FID with signal to noise ratio greater than the dynamic range of the computer memory cell into which it is being time averaged. When the largest value in a time averaged FID overflows the computer memory cell being used to store it, the data must be scaled in order to continue time averaging; but, for each factor of two by which the data is scaled, one bit of the ADC becomes useless decreasing the effective dynamic range of the ADC by a factor of two. When this process reaches a point where the least significant effective bit of the ADC represents a value larger than the noise level, no amount of additional time averaging can bring additional small signals out of the noise.

In Section 6 we learned about T_1 relaxation. Let us examine what effect longitudinal relaxation has on time averaging of FID's. Suppose we apply a θdegree pulse to a spin system every *t* seconds, collect a FID after each pulse, and time average *N* such FID's in a total time of $T_o = Nt$. Starting from thermal equilibrium, the amplitude of the first FID will be proportional to $M_o \sin\theta$, but, if t is short enough so that the magnetization does not fully relax, the magnetization available to generate the second FID will be smaller and for the

third FID still smaller and so on. A steady state will be reached after a few pulses characterized by Equation (30).

$$\begin{aligned} M^+ &= M^- \cos\theta \\ M^- &= M_o + \left(M^+ - M_o\right)e^{-r} \end{aligned} \tag{30}$$

where

M^+ = the *z* axis magnetization just after the pulse

M^- = the *z* axis magnetization just before the pulse

M_o = the thermal equilibrium magnetization

r = t/T_1

Substituting the first line of Equation (30) into the second and solving for M^- gives Equation (31).

$$M^- = M_o \frac{e^r - 1}{e^r - \cos\theta} \tag{31}$$

The *xy* magnetization following the pulse is $M^- \sin\theta$, and the amplitude of the FID is proportional to this quantity. Hence, the signal amplitude for each FID can be rewritten for this case as Equation (32).

$$S(\theta, r) = KM^- \sin\theta = KM_o \sin\theta \frac{e^r - 1}{e^r - \cos\theta} \tag{32}$$

Then, we know from Equation (29) that the signal-to-noise after *N* FID's is proportional to $\sqrt{N}$. Hence, we can write Equation (33).

$$\bar{S}_t(\theta, r) = \frac{\sqrt{N}}{\sigma_S} S(\theta, r) = \frac{KM_o\sqrt{N}}{\sigma_S} \sin\theta \frac{e^r - 1}{e^r - \cos\theta} \tag{33}$$

However, the interesting question is: What values of θ and r will give maximum signal-to-noise per unit time? To answer this we write Equation (34).

$$\begin{aligned}\bar{S}_t'(\theta,r) &= \frac{\bar{S}_t(\theta,r)}{T_o} = \frac{KM_o\sqrt{N}}{\sigma_S T_o}\sin\theta\frac{e^r-1}{e^r-\cos\theta} \\ &= \frac{KM_o}{\sigma_S\sqrt{T_oT_1}}\sin\theta\frac{e^r-1}{\sqrt{r}e^r-\cos\theta} \\ &= K'\sin\theta\frac{e^r-1}{\sqrt{r}e^r-\cos\theta}\end{aligned} \tag{34}$$

The explicit dependence on N has been eliminated by using the fact that

$$N = \frac{T_o}{t} = \frac{T_o}{T_1 r}$$

and all of the constants have been absorbed into a new constant by defining

$$K' = \frac{KM_o}{\sigma_S\sqrt{T_oT_1}}$$

Equation (34) tells us how signal-to-noise per unit time depends on the values of θ and r. Figure 17 shows a contour plot of this equation. Note that 90 degree pulses never give maximum signal-to-noise per unit time regardless of the repetition rate.

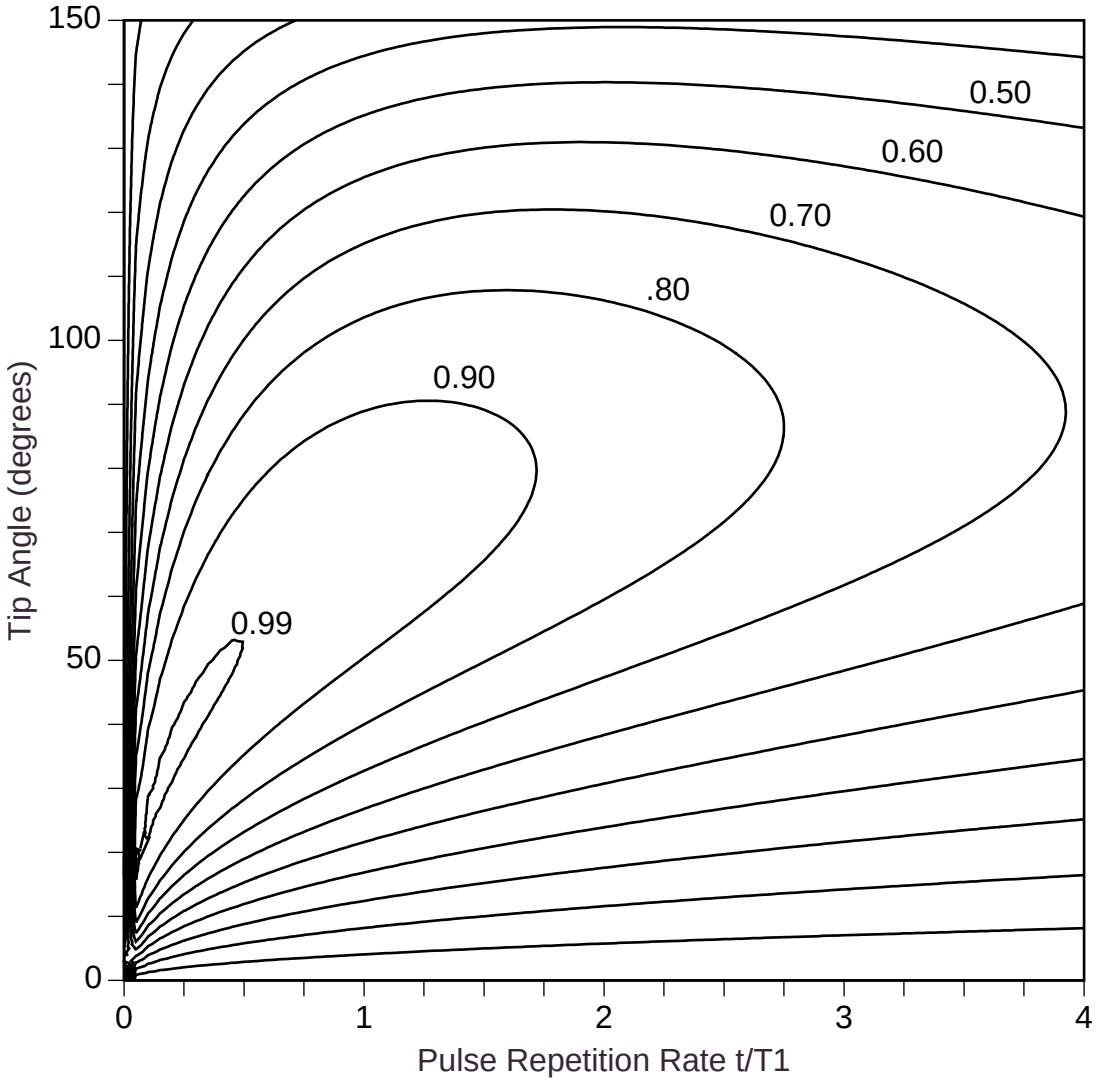

150
100
50
0
0
1
2
3
4
Tip Angle (degrees)
Pulse Repetition Rate t/T1
0.50
0.60
0.70
.80
0.90
0.99

Figure 17

10. Digital Filters and Convolution.

In the context of NMR spectroscopy digital filters are computational algorithms that reject unwanted signals or noise and allow desirable signals to pass through to the final spectral display. Filters can also alter line shapes with either desirable or undesirable results. These undesirable results are generally caused by unintentional digital filtering.

10.1. The Convolution Theorem.

In preparation for discussion of digital filters it is necessary to understand the Fourier convolution theorem. Suppose one has three functions of time, $a(t)$, $b(t)$, and $c(t)$, such that,

$$c(t) = a(t)b(t) \tag{35}$$

then, if $A(\omega)$, $B(\omega)$, and $C(\omega)$, are the Fourier transforms of each of the corresponding time functions, it can be shown that

$$C(\omega) = \int_{-\infty}^{\infty} \mathrm{d}\,\omega' A(\omega)B(\omega - \omega') \tag{36}$$

Of course, these computations must be done on sampled functions as has been discussed previously. Figure 18 illustrates how the convolution operation works in the frequency domain for two lines of equal width. In the computer the calculation would actually be done by multiplying the FID's corresponding to the two lines together and transforming the product to give the convolved spectrum. Due to the computational efficiency of the FFT, this can be done far faster than computing the convolution directly. The former takes only $2N\log_2 N$ operations, whereas, the latter requires N^2 operations. This increase in computational efficiency is the power of the convolution theorem.

> Problem 21: Compute the increased computational efficiency of the FFT method over direct computation for convolution of two 1K (1024) point data tables and for two 32K (32,768) point data tables.

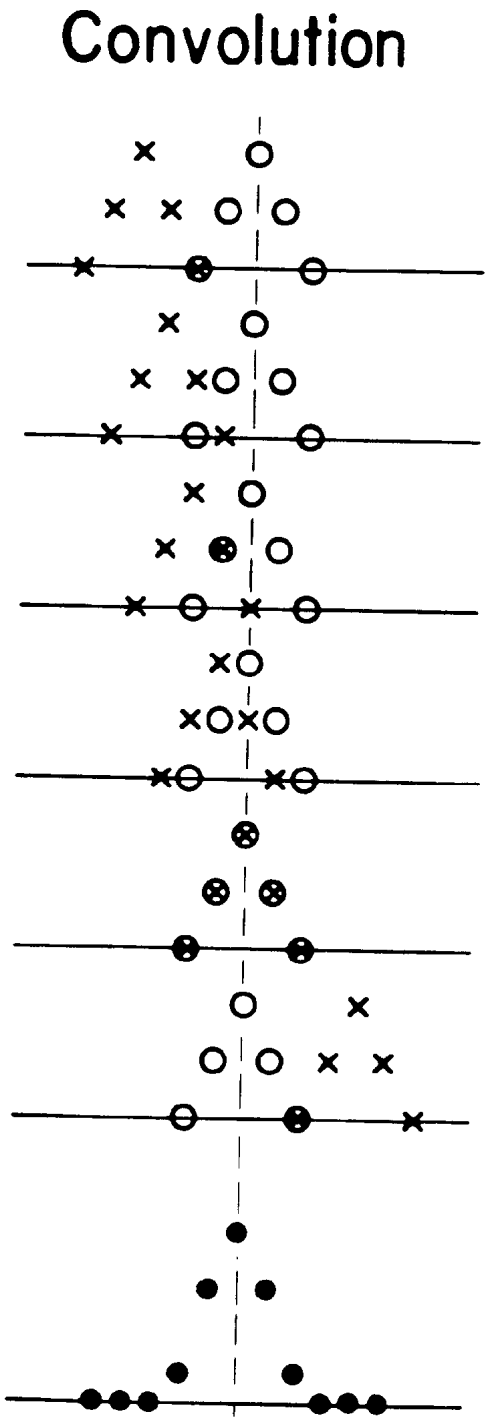


Figure 18

10.2. Deconvolution.

If a spectrum has been convolved with some particular function, (perhaps due to an artifact of the instrumentation, such as, magnet inhomogeneity) it is possible to conceive of undoing the process by writing

$$a(t) = \frac{c(t)}{b(t)} \tag{37}$$

This is, indeed, possible; however, certain limitations apply since one must avoid dividing by zero; and, of course, it is impossible to recover data lost because the original $c(t)$ was zero over some particular interval.

10.3. Truncation of the FID.

If one stops taking data before the FID has completely died away into the noise, this can be viewed as convolution. The FID has been multiplied by a function of the form

$$f(t) = \begin{cases} 0 & t < 0 \\ 1 & 0 \le t \le A \\ 0 & t > A \end{cases} \tag{38}$$

where A is the spectrometer acquisition time. This corresponds to convolving the spectrum with a $(\sin x)/x$ function. (See Figure 19.) The $(\sin x)/x$ function is the Fourier transform of the rectangular function Equation (38).

10.4. Apodization.

The effects of this undesirable convolution can be alleviated by a technique called apodization. This consists of smoothing the tail of the FID so as to eliminate the sharp edge produced by the rectangle function. Almost any smooth function will produce desirable effects; one method is shown in Figure 19.

10.5. Sensitivity Enhancement.

An FID can be written in the form

$$f(t) = \exp\left(-i\omega t - t/T_2\right) \tag{39}$$

Recall that the width of the line when one Fourier transforms this function is given by Equation (25). Suppose the above function is multiplied by one of the form

$$f_c(t) = \exp\left(-t/T_{LB}\right) \tag{40}$$

Problem 22: Show that the complex exponential form of the FID Equation (39) is just another way of writing Equation (22). Show that multiplying Equation (39) by Equation (40) gives, in the spectrum, a Lorentzian line with frequency, ω, and width,

$$L = \frac{1}{\pi T_2} + \frac{1}{\pi T_{LB}} \tag{41}$$

corresponding to the convolution of the two Lorentzians.

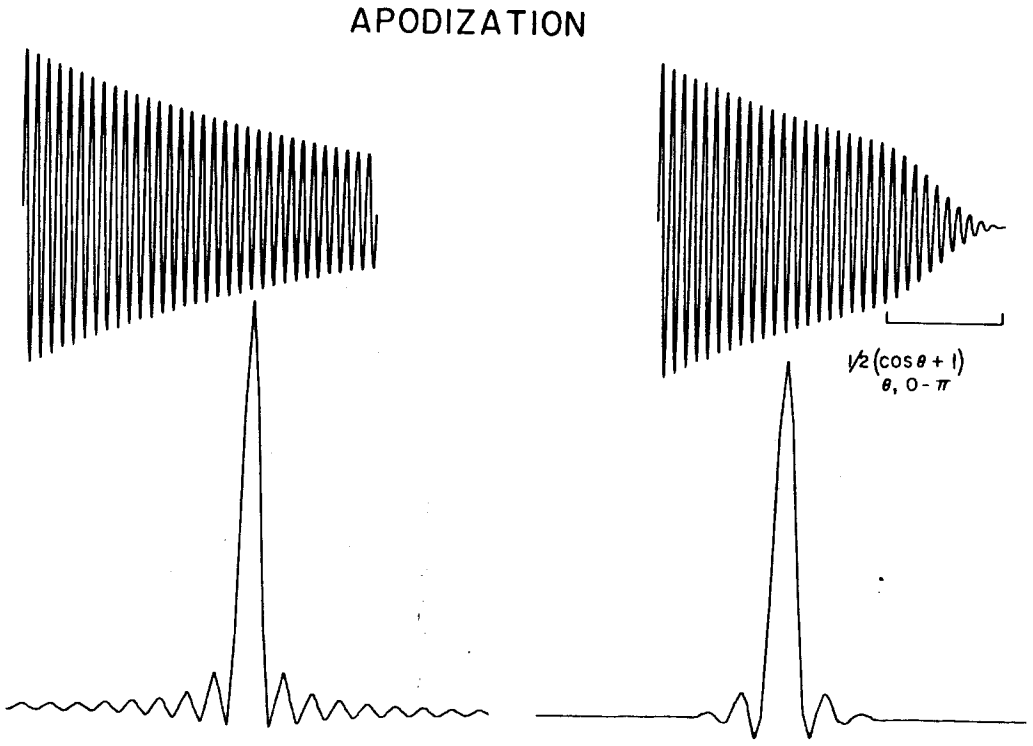


Figure 19

The line width is increased by $1/\pi T_{LB}$ which decreases the resolution, but, as shown in Figure 20, the noise in the spectrum is substantially decreased, thus, improving the signal to noise ratio of the spectrum.

10.6. Resolution Enhancement.

If the sign of the exponential is changed in the convolution function to give

$$f_c(t) = \exp(+t/T_{LB}) \tag{42}$$

then, by the same arguments given above, the line width will be decreased. However, just as the signal to noise ratio was increased before, it will now be decreased.

Problem 23: Show that resolution enhancement is an example of deconvolution as defined above. Hint: What is the reciprocal of an exponential?

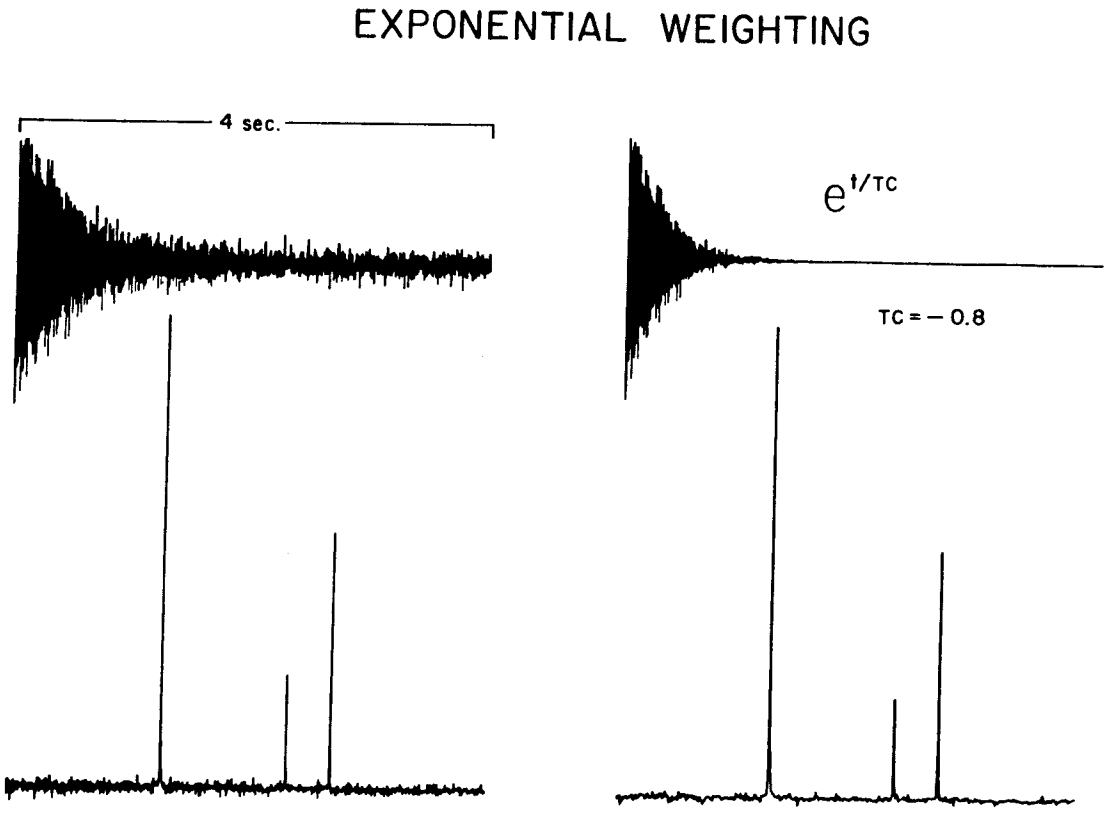


Figure 20

10.7. Lorentzian to Gaussian Transformation.

Consider a convolution function of the form

$$f_c(t) = \exp\left(+t/T_{LB} - t^2/T_G^2\right) \tag{43}$$

This corresponds to simultaneous convolution with a Gaussian and deconvolution with a Lorentzian. In an ideal situation this will convert a Lorentzian line into a Gaussian line. This is a technique commonly used in 2D NMR.

11. Decoupling and the Nuclear Overhauser Effect.

Thus far our discussion has centered mainly on the properties of a single spin-1/2, but spins are not, in general, isolated. They interact with each other in various ways, among which are scalar coupling and nuclear dipole-dipole interactions. Decoupling consists of irradiating a system of interacting spins continuously and observing the effect on the spectrum. Decoupling is termed heteronuclear if the observed and irradiated nuclei are different nuclear species, e.g., one can observe ^{13}C while irradiating ^{1}H; or homonuclear if they are the same, e.g., one can irradiate a particular proton while observing the effect on the rest of the proton spectrum. A commonly used notation for these two experiments is ^{13}C{^{1}H} and ^{1}H{^{1}H} respectively.

The term decoupling stems from the fact that, when a spin is irradiated, the fine structure or splittings of all the other spins in the coupling network which are due to scalar coupling with the irradiated spin are collapsed, so that the splitting pattern appears as if the irradiated spin were not coupled to the others. The intensities of the remaining resonances, however, may be substantially different from those expected in a spin system created by removing the irradiated spin and considering only the remaining spins. These intensity differences are called the nuclear Overhauser effect (NOE). The NOE arises as a consequence of the T_1 type relaxation occurring in the spin system. As an example of these principles let us examine the equation of motion for a proton coupled to a carbon-13 nucleus.

11.1. Equation of Motion for a Two Spin System.

Figure 21 shows (a) the energy level diagram of a single spin-1/2 with only one relaxation pathway available in contrast to (b) a system of two spins-1/2 with four energy levels and six possible relaxation pathways. Using the density matrix formalism, one can show that the z-components of the magnetization evolve according to

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} M_z^C(t) \\ M_z^H(t) \end{pmatrix} = -\begin{pmatrix} \rho_C & \sigma \\ \sigma & \rho_H \end{pmatrix}\begin{pmatrix} M_z^C(t) - M_z^C(\infty) \\ M_z^H(t) - M_z^H(\infty) \end{pmatrix} \tag{44}$$

where

$$\rho_{\mathrm{C}} = \frac{1}{T_{1\mathrm{C}}} = W_2 + 2W_{1\mathrm{C}} + W_0 \tag{45}$$

$$\rho_{\mathrm{H}} = \frac{1}{T_{1\mathrm{H}}} = W_2 + 2W_{1\mathrm{H}} + W_0 \tag{46}$$

and

$$\sigma = \frac{1}{T_{1\mathrm{CH}}} = W_2 - W_0 \tag{47}$$

Also note that

$$M_z^{\mathrm{H}}(\infty) = \frac{\gamma_{\mathrm{H}}}{\gamma_{\mathrm{C}}} M_z^{\mathrm{C}}(\infty) \tag{48}$$

If the proton is strongly irradiated, the scalar coupling manifested in the carbon spectrum disappears immediately; and, after several T_1's, the proton transitions saturate, i.e., $M_z^{\mathrm{H}} \to 0$.

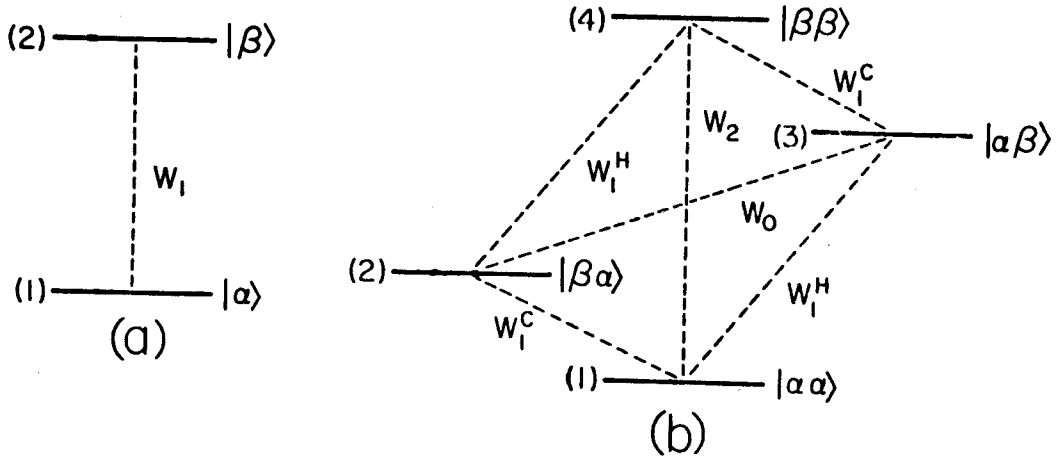


Figure 21

Problem 24: Show that under decoupling the steady state solution of the equation of motion is

$$M_z^C = \left(1 + \eta_{C\{H\}}\right) M_z^C(\infty) \tag{49}$$

where

$$\eta_{C\{H\}} = \frac{\gamma_H \sigma}{\gamma_C \rho_C} \tag{50}$$

Under proton decoupling the equation of motion for the ^{13}C magnetization becomes

$$\frac{\mathrm{d}\, M_z^{\mathrm{C}}(t)}{\mathrm{d}\, t} = -\rho_{\mathrm{C}}\left[M_z^{\mathrm{C}}(t) - \left(1 + \eta_{\mathrm{C\{H\}}}\right) M_z^{\mathrm{C}}(\infty)\right] \tag{51}$$

This means that the ^{13}C magnetization evolves just as though the proton is not there except that it evolves toward the NOE enhanced value rather than toward the usual thermal equilibrium. If the relaxation is purely dipolar, then $\rho_{\mathrm{C}} = 2\sigma$, and $\eta = 2$.Thus, the carbon magnetization will be enhanced by a factor of 1 + η = 3. If other relaxation mechanisms are important, the factor will be less than three. It is the factor, $1 + \eta$, which is generally referred to as the NOE.

The same principles apply to other nuclear pairs besides protons and carbon-13; but note that, if the gyromagnetic factors have opposite sign as would be the case for protons and nitrogen-15, then the NOE would be negative, and line intensities would be decreased from their thermal equilibrium values even becoming zero or negative.

Problem 25: What would be the NOE for a ^{15}N{^{1}H} experiment under purely dipolar relaxation? Draw a schematic ^{15}N spectrum of formamide showing intensities to scale with and without proton decoupling. Ignore the long-range coupling to the formyl proton.

11.2. Homonuclear Decoupling.

The discussion above applies equally well to a system of two nonequivalent protons.

Problem 26: What would the maximum NOE be for two protons?

Since the dipolar relaxation mechanism depends critically on the through space distance between the interacting nuclei, it is possible in certain cases to determine internuclear distances using proton-proton NOE's. This technique is coming to be widely used for determining structure in macromolecules such as proteins.

11.3. Broadband Decoupling.

The usual way to run ^{13}C spectra is with all the protons decoupled. To accomplish this it is, in general, necessary to simultaneously irradiate protons of varying chemical shifts. This is done by modulating the decoupler rf transmitter so as to spread the power over the necessary band of frequencies. For a number of years this was done by using pseudo random noise for modulation, but more recently other techniques have been developed which use the available power more effectively. The current best such technique for $^{13}C\{^{1}H\}$ experiments seems to be the WALTZ modulation sequence of Freeman. It is important to minimize the amount of power used for broadband decoupling. Since the power is on continuously, it can heat the sample in much the same way as a microwave oven heats food. Using WALTZ modulation it is generally possible to decouple over the full proton chemical shift range using power levels of the order of one watt in a five or ten millimeter sample.

12. Multiple Pulse Experiments, Spin Echoes, Measurement of T_1 and T_2.

In the preceding section the consequences of subjecting the nuclear spins to a single radio frequency pulse were outlined, but the great variety of data available from NMR experiments is obtained principally from subjecting the spins to multiple pulses of various phases and amplitudes separated by carefully chosen delays. Many of the principles involved can be illustrated using a simple two pulse sequence to measure the value of T_1 and T_2.

12.1. Measurement of T_1 by Inversion Recovery.

We have seen previously, Equation (16), how the *z* component of the magnetization relaxes exponentially back to thermal equilibrium whenever it is disturbed from that state. Figure 22 shows a pulse sequence which permits quantitative measurement of this process by producing a series of partially relaxed spectra. By varying the time t_1 between the 90 and 180 degree pulses the partially relaxed *z* magnetization is projected on the *y*-axis. Fourier transformation of the resulting FID and measurement of the peak intensities then produces a set of data for each peak in the spectrum which can be analyzed to obtain the value of T_1. (See Equation (55).)

Problem 27: We have considered previously (Figure 7) the inadequacy of a 180 degree pulse when $\mathbf{B}_{eff}$ differs appreciably from $\mathbf{B}_1$, i.e., when the line is too far off resonance. If the 180 degree pulse is replaced by

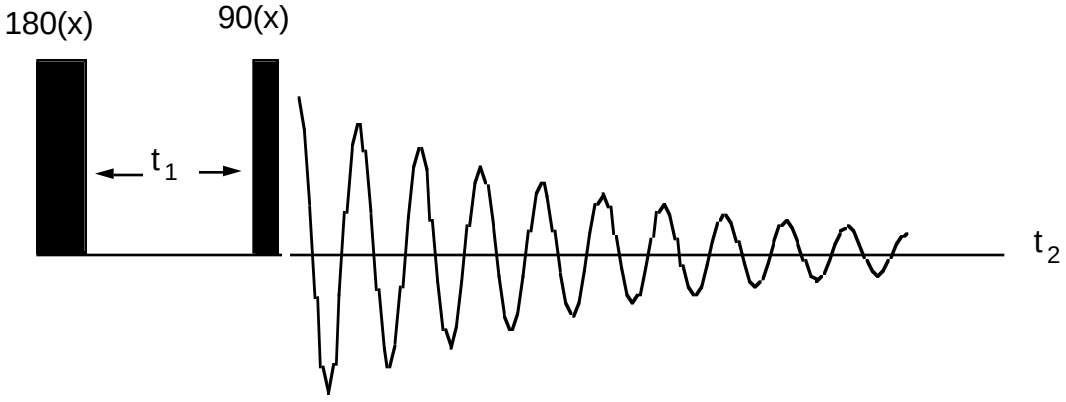


Figure 22

a “pulse sandwich” of the form 90(*x*)180(*y*)90(*x*), describe how this situation will be improved.

12.2. Hahn Spin Echoes.

Figure 23 shows another pulse sequence which permits the measurement of T_2. At 2τ, dephasing due to magnet inhomogeneity is reversed and the magnetization in said to refocus or form a spin echo. The concept of refocussing of magnetization is critical to an understanding of two dimensional experiments. True T_2 processes, which are stochastic or random in nature, do not refocus; so the amplitude of the magnetization is reduced but only by these stochastic processes. The second half of the echo can be Fourier transformed to produce a spectrum in the usual way. The value of T_2 can be extracted from peak intensities measured from a series of such spectra taken as a function of t_1 in much the same way as was described for the T_1 measurement.

12.3. Measurement of T_2 by the Carr, Purcell, Meiboom, Gill Method.

If the spins diffuse into a region of different magnetic field during t_1, this constitutes a stochastic process; then the refocussing will not accurately represent spin-spin relaxation processes. This problem can be largely overcome by the pulse sequence of Figure 24. The series of 180(*y*) pulses repeatedly refocuses the magnetization. In this case only the diffusion happening during 2τ effectively reduces the echo amplitude. By making τ short enough, the effects of diffusion can be made insignificant. The second half of each echo can be taken as an FID, Fourier transformed, and analyzed as before. With the pulse se-

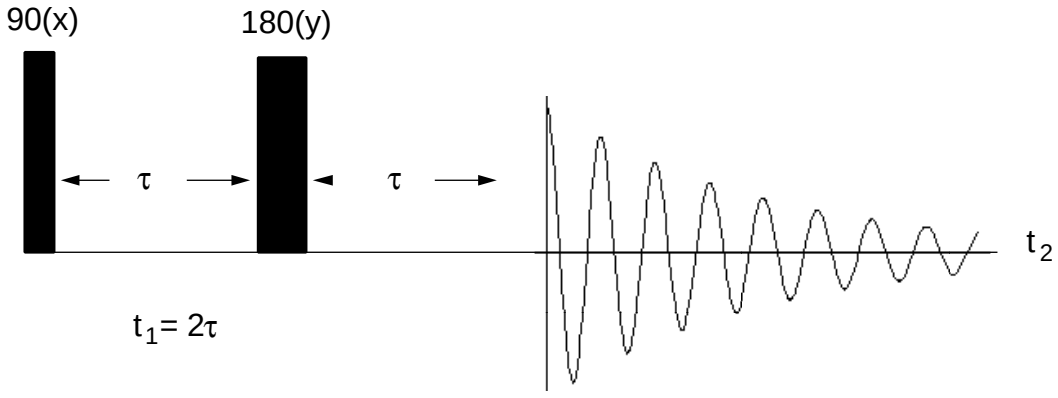


Figure 23

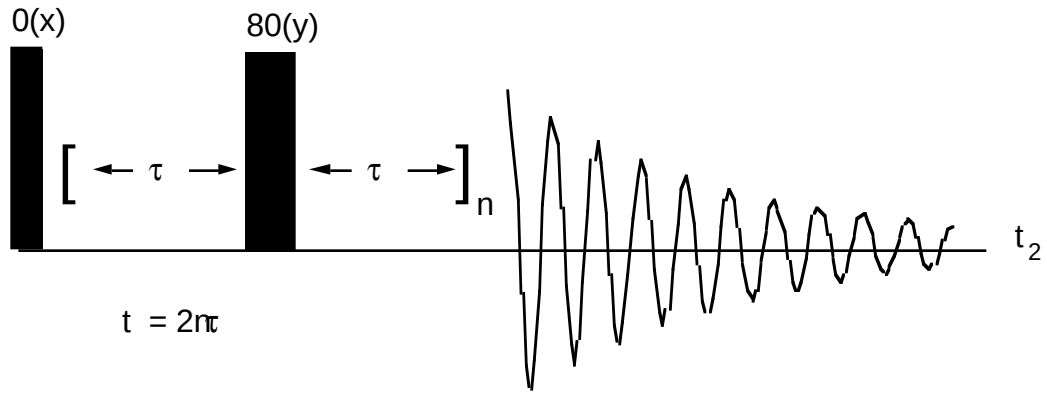


Figure 24

quence as written in Figure 24, pulse imperfections in the 180 degree pulses tend to accumulate. But, merely by alternating the phase of successive y pulses this can largely be eliminated.

Problem 28: This experiment will work also if the train of 180 degree pulses is not phase shifted with respect to the initial 90 degree pulse. How will the results of the experiment differ in this case? Why is this not a better way to measure T_2?

13. Polarization Transfer, INEPT and DEPT.

The magnetization generated in a sample depends on the γ of the nucleus involved, so a high γ nucleus, such as ^{1}H, develops greater magnetization in a constant field than, for example, ^{15}N or ^{13}C. If a high γ nucleus and a low γ nucleus are coupled by scalar coupling, magnetization can be transferred from the high to the low γ nucleus thus increasing the sensitivity with which the low γ nucleus can be detected.

13.1. INEPT.

Figure 25 shows the pulse sequence used for the INEPT (Insensitive Nucleus Enhanced by Polarization Transfer) experiment. Vector diagrams show the evolution of the magnetization at key points. The principle problem with this method is that antiphase multiplets are generated which cancel to produce zero intensity when decoupling is applied. An additional refocussing step can be added to the pulse sequence to overcome this problem, but the DEPT pulse sequence also solves this problem and has other positive attributes as well.

13.2. DEPT.

Figure 26 shows the DEPT (Distortionless Enhancement by Polarization Transfer) pulse sequence and vector diagram. Since DEPT depends on the properties of double quantum coherence to achieve its effect, the vector diagrams are not completely satisfactory, but do provide some sense of what is happening to the magnetization. Scalar coupled multiplets produced by DEPT are enhanced but have the same intensity ratios as a normal FT spectrum. Thus, they can be easily interpreted or decoupled to produce a simpler spectrum in the usual way. By adjusting the width, θ, of the final proton pulse, one can produce spectra which identify various types of spin systems. Figure 27 shows how CH, CH_2, and CH_3 spin systems respond to the tip angle of the final proton pulse with all other parameters of the pulse sequence kept constant. For $\theta = 90$ degrees only methine carbons appear in the spectrum. For θ = 135 degrees methine and methyl carbons are positive while methylene carbons are negative. Of course, quaternary carbons never appear because there is no source of polarization for them. These two spectra plus an ordinary proton decoupled FT spectrum to pick up the quaternary carbons provide sufficient information to determine the number of directly bonded protons for each carbon in the spectrum. One additional DEPT spectrum with θ = 45 degrees yields three independent pieces of information, so that appropriate linear combinations of the three spectra can yield subspectra containing only methine, meth-

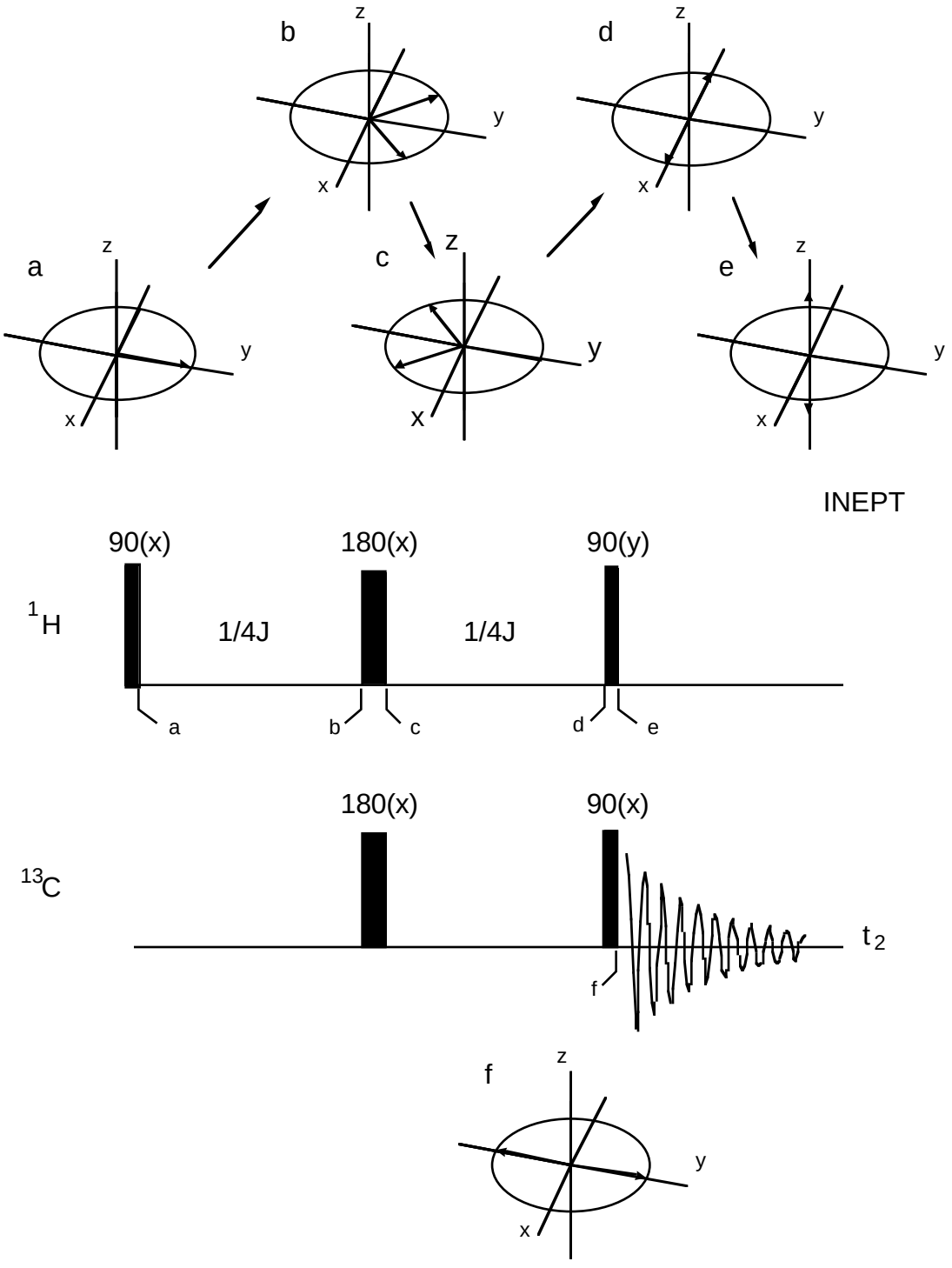


Figure 25

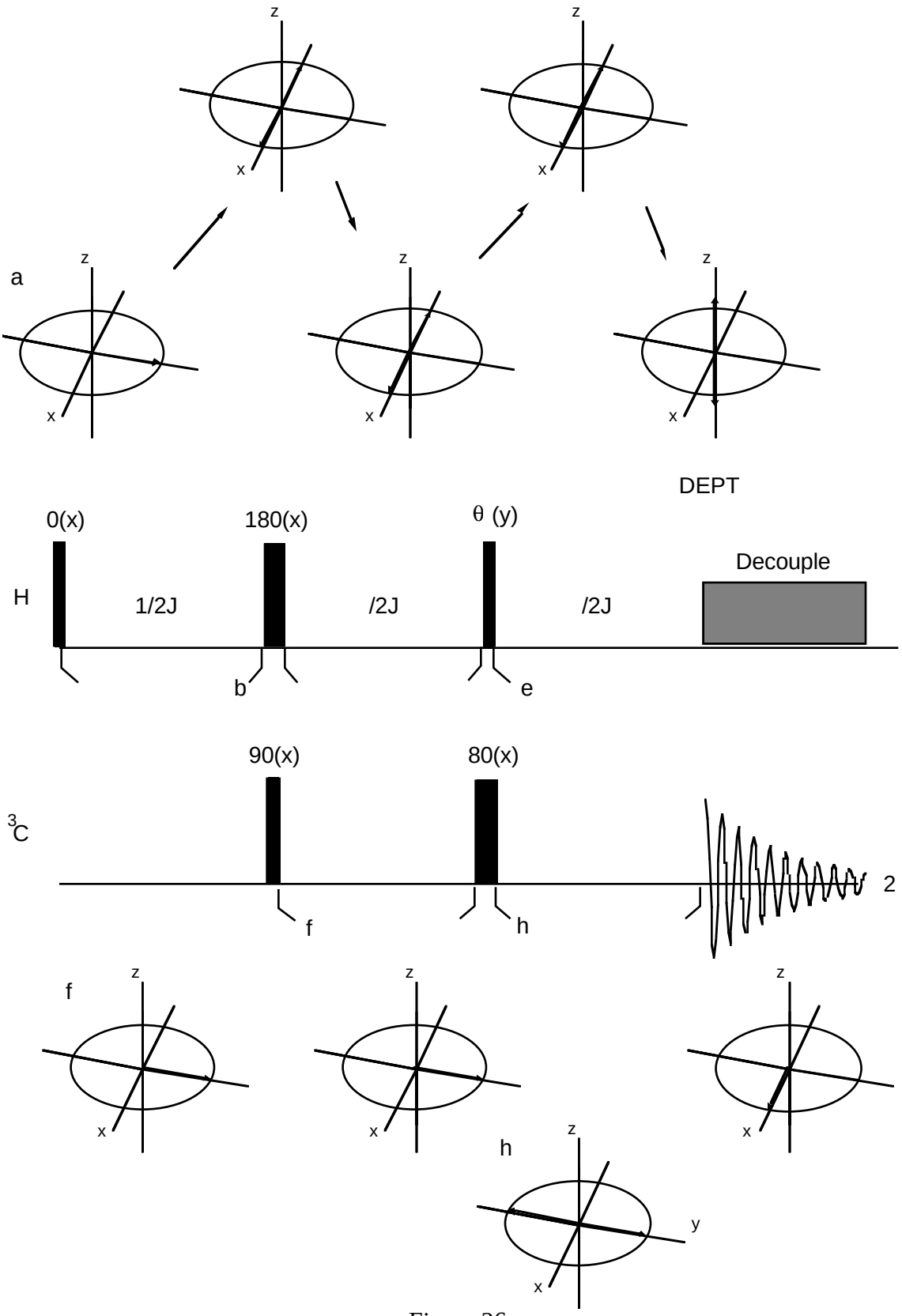


Figure 26

ylene, or methyl resonances. An example of this treatment is shown in Figure 28 for menthol.

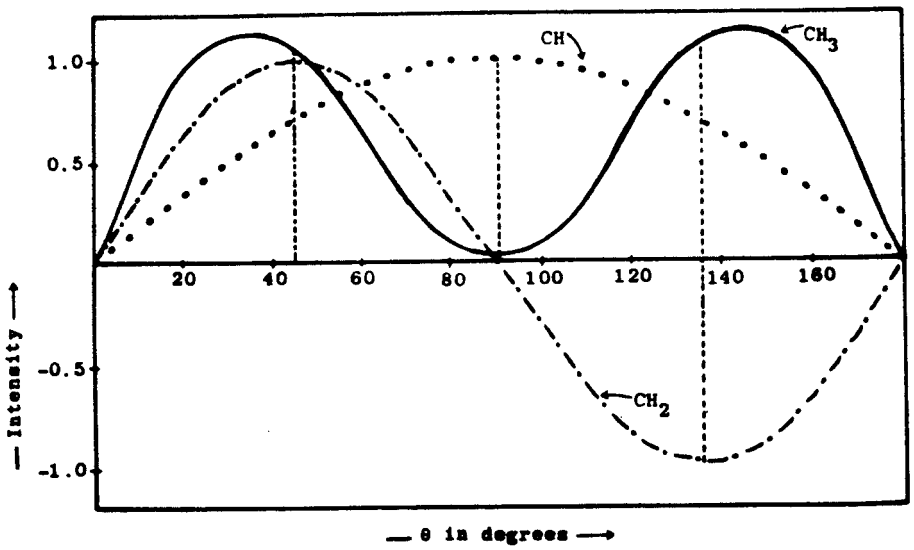


Figure 27

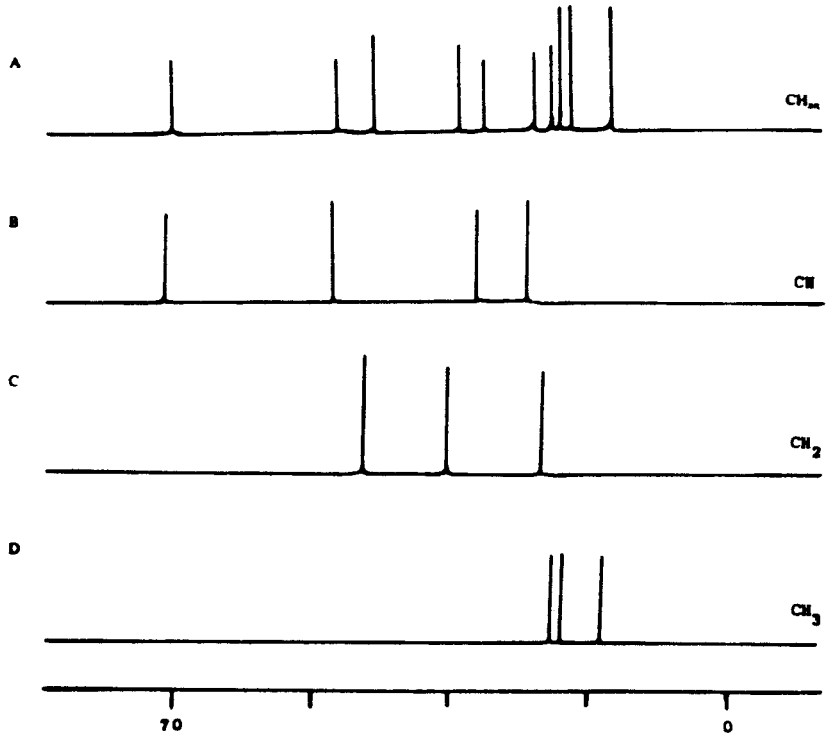


Figure 28

The DEPT experiment can also be used as a convenient method for calibrating the proton pulse width. Usually, in experiments of this type, proton pulses are applied using the decoupler transmitter. Since the decoupler pulses, $\mathbf{B}_2$, essentially always have a different calibration factor than the observe pulses, $\mathbf{B}_1$, it is necessary to have an indirect method of calibrating $\mathbf{B}_2$ with the instrument set up as it will be when the experiments which will use the calibration are done. This can be accomplished by observing a methylene resonance using a DEPT pulse sequence with θ set to nominally 90 degrees. Then, adjusting the proton pulse width, one can observe the methylene resonance to pass through a null as the proton pulse passes through exactly 90 degrees. If the calibration point is completely unknown, a rough calibration can be achieved by setting θ to a nominal 45 degree value and searching for a maximum intensity as a function of the proton pulse width. This calibration can be used in numerous other one and two dimensional experiments where decoupler pulses are required.

14. Fundamental Concepts of Two Dimensional NMR.

Before discussing specific 2D experiments some fundamental concepts characteristic of all 2D experiments will prove useful.

14.1. The Generic 2D Pulse Sequence.

Four time periods characterize a 2D experiment as shown in Figure 29. Each of the four periods can take a variety of forms depending on the experiment, but some fairly typical examples can be given. Preparation frequently consists of just waiting long enough for the spins to be sufficiently close to thermal equilibrium or some steady state condition then, perhaps, applying a 90 degree pulse to produce transverse magnetization or coherence. Thus prepared, the magnetization is allowed to evolve, often with some refocussing pulses, so as to exhibit some desired characteristic of the spin system as a function of t_1. The mixing period can be used to enhance certain aspects of the evolving magnetization, i.e., to filter out just the information the experimenter wants and convert it into the single quantum coherence which can be detected by the spectrometer. During detection the carefully prepared transverse magnetization is sampled as a function of t_2. Systematic incrementation of t_1 with sampling of the resulting transverse magnetization as a function of t_2 for each t_1 increment, yields a two dimensional FID as illustrated in Figure 30. The form of the 2D FID is

$$f(t_1,t_2)=\exp\left(-i\omega_i t_1-\frac{t_1}{T_{2,1}}\right)\exp\left(-j\omega_j t_2-\frac{t_2}{T_{2,2}}\right). \tag{52}$$

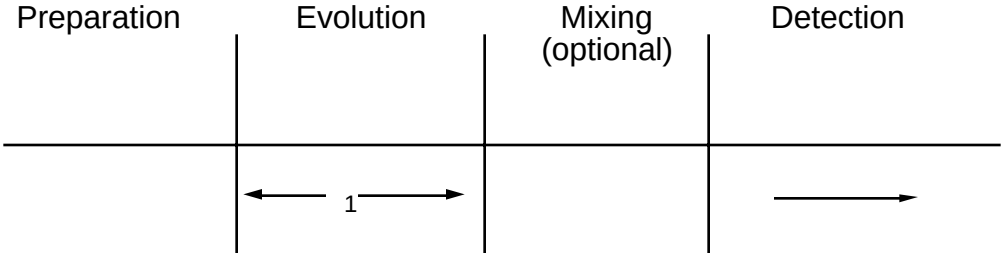


Figure 29

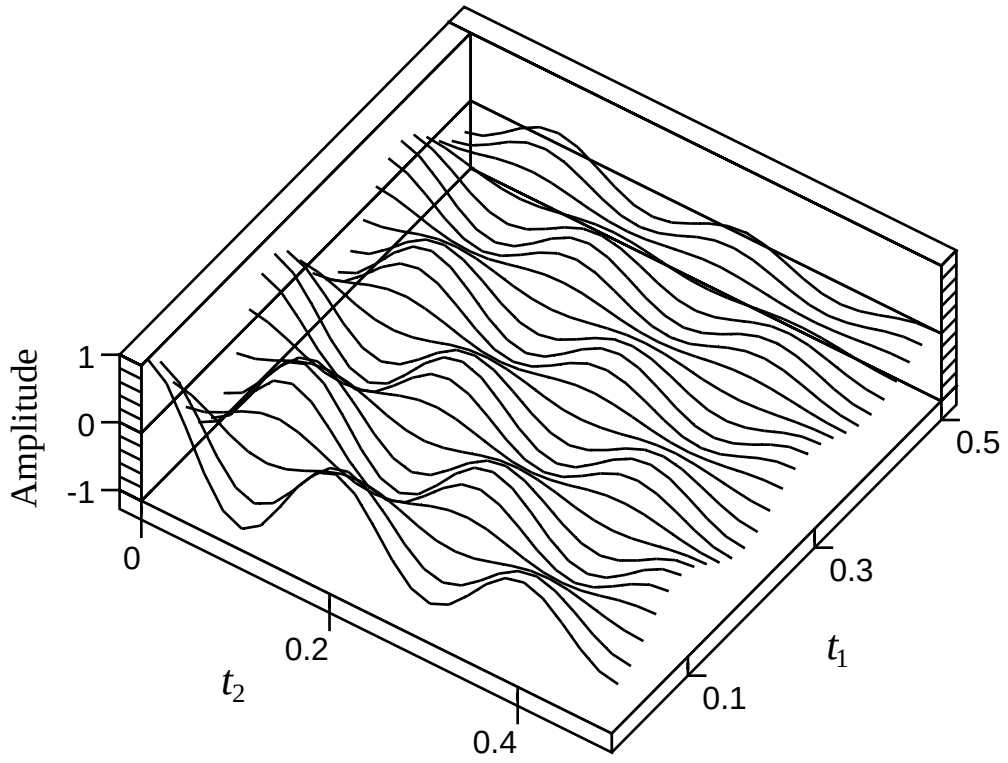


Figure 30

The function *f* is called hypercomplex because it is complex in both the t_1 and t_2 dimensions. The imaginary numbers *i* and *j* are defined such that $i^2 = -1$, and $j^2 = -1$, but $ij \neq -1$. Thus, Equation (52) involves four types of terms: those involving neither *i* nor *j*, those involving *i*, those involving *j*, and those involving the product *ij*. These terms are often referred to as real-real, imaginary-real, real-imaginary, and imaginary-imaginary respectively. Figure 30 shows the real-real part of *f*. This will be discussed further in Section 14.3.

14.2. The Two Dimensional Fourier Transform.

The FID must now be subjected to a 2D Fourier transform. First, each of the FID's is transformed with respect to t_2 applying any convolution functions desired. This produces a matrix which is a function of t_1 and ν_2 as shown in Figure 31. The time functions in the t_1 domain have the appearance of FID's, but in the literature they are often called interferograms. Each interferogram is now transformed with respect to t_1 after

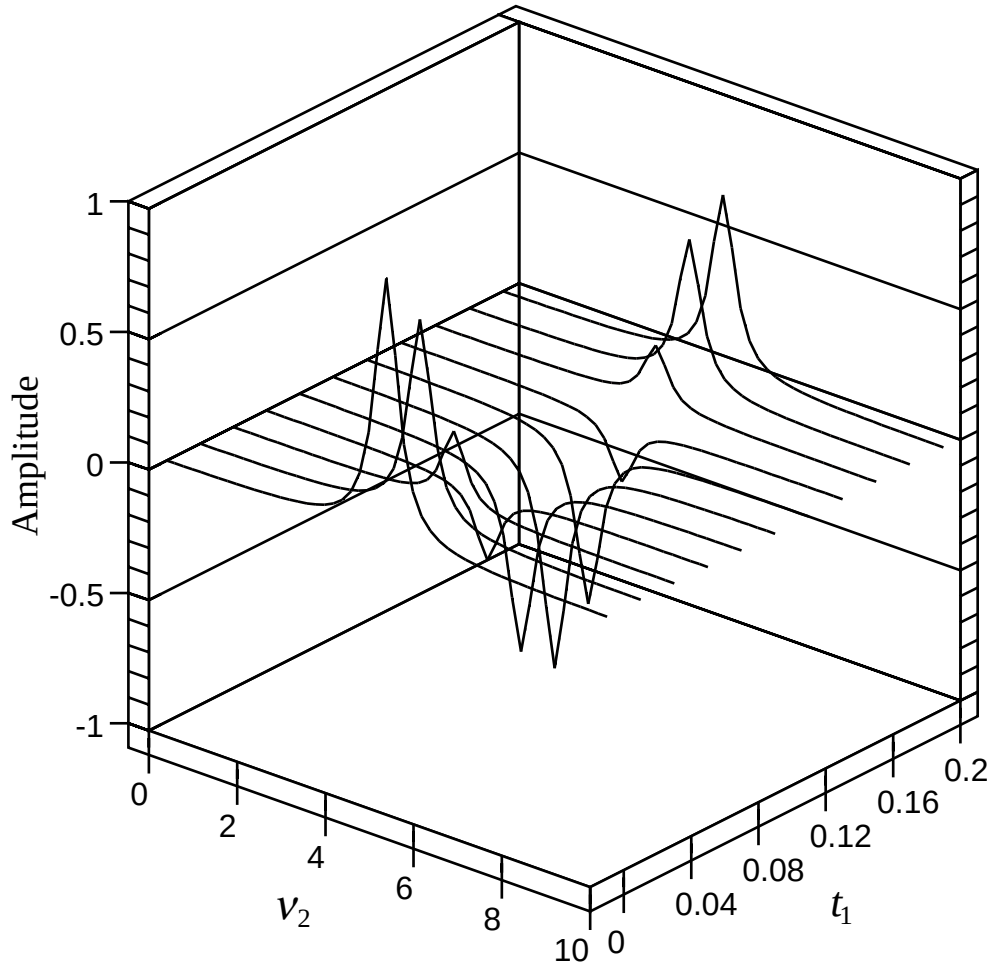


Figure 31

applying any convolution functions. The result is then a two dimensional Lorentzian line, a function of ν_1 and ν_2, modified by any convolutions as shown in Figure 32.

14.3. Phase Sensitive 2D NMR.

Figure 32 shows a pure absorption line shape in both dimensions; however, many 2D experiments do not yield this result but rather what is called a phase twist line shape as shown in Figure 33. This line shape has strong tails and a much wider appearance due to the dispersion contribution. Figure 31 shows pure amplitude modulation of the ν_2 spectra as a function of t_1. This phase twist line shape results from phase modulation as a function of t_1. as shown in Figure 34. Generally the pulse sequences that produce a phase twist line shape are those that are designed to achieve quadrature detection in the t_1 dimension. Spectra with this phase twist line shape are normally displayed as the absolute value of the hy-

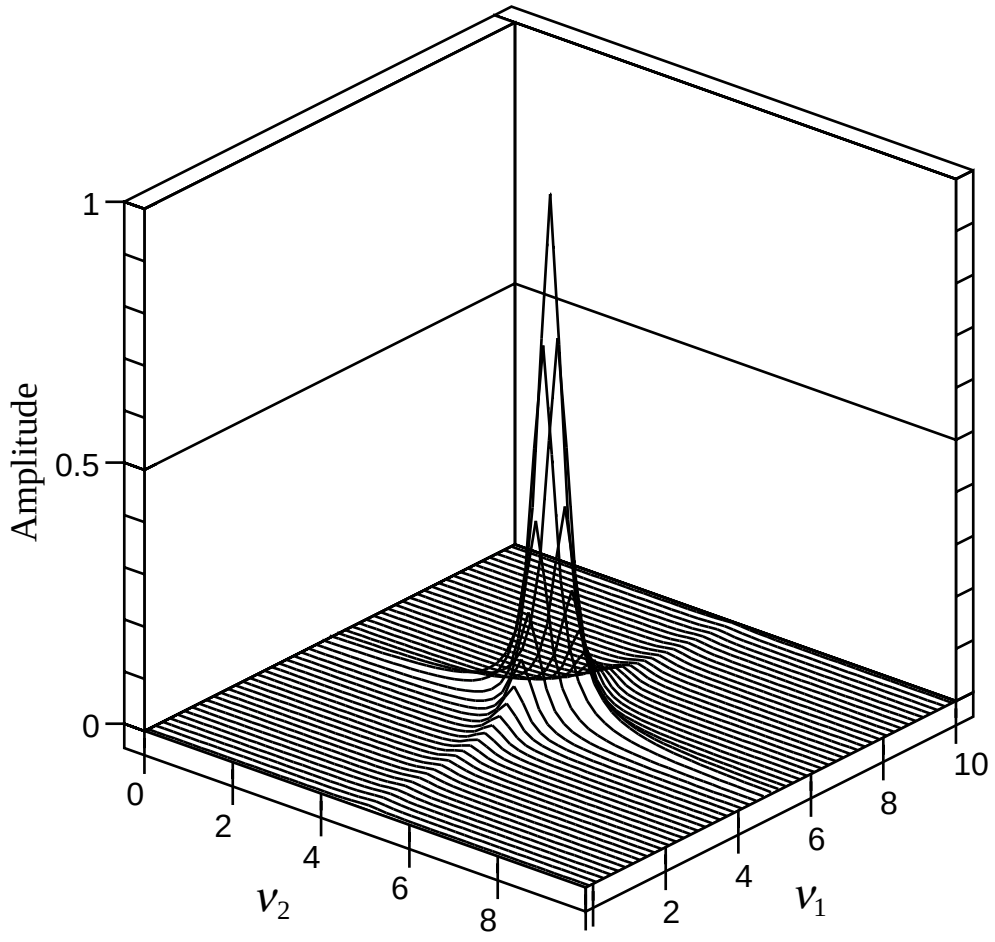


Figure 32

percomplex quantities. An alternative method first published by States, Haberkorn, and Ruben makes use of two data sets to produce a spectrum that can be displayed as a two-dimensional pure absorption mode spectrum with lines as in Figure 32. In the time domain one data set is amplitude modulated like that shown in Figure 30, while the second is similar, but the amplitude modulation with respect to t_1 is 90 degrees out of phase as shown in Figure 35. One then has four data sets or quadrants as mentioned in Section 14.1 which can be transformed to yield the corresponding hypercomplex spectrum. Phasing of such a spectrum is somewhat more complex than for a one-dimensional spectrum since linear combinations of all four quadrants of the hypercomplex data are required.

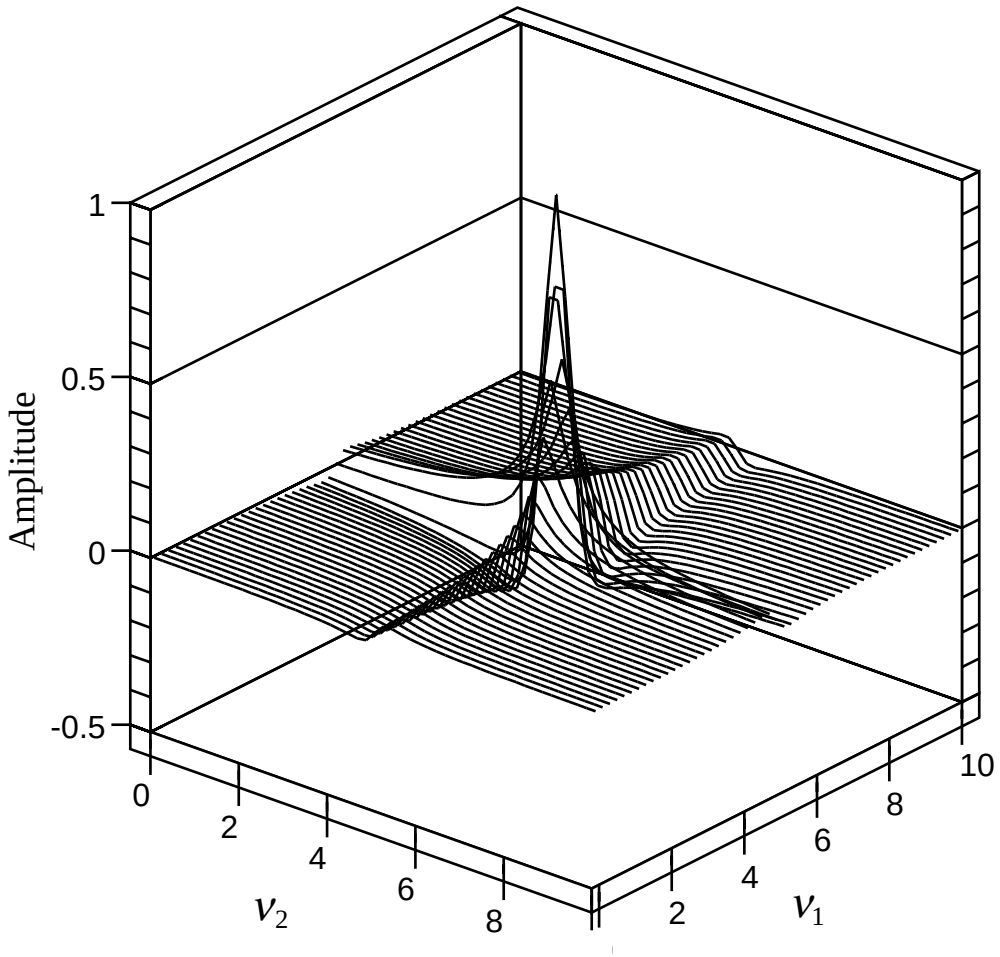


Figure 33

14.4. Phase Cycling.

Phase cycling is used in both one and two dimensional spectroscopy to cancel artifacts and unwanted signals while retaining the information which the experimenter wants. The basic idea is to create a series of FID's, such that, the unwanted coherences alternate in phase while those to be kept have always the same phase. Thus, when the FID's are added together, the unwanted parts interfere destructively, but the desired parts interfere constructively. Simultaneously, of course, the signal to noise improves as we have discussed previously. However, whether or not the signal to noise improvement is needed, some minimum number of FID'S must be acquired to permit the phase cycle to complete. This number may be as small as two or as large as 1024 or more.

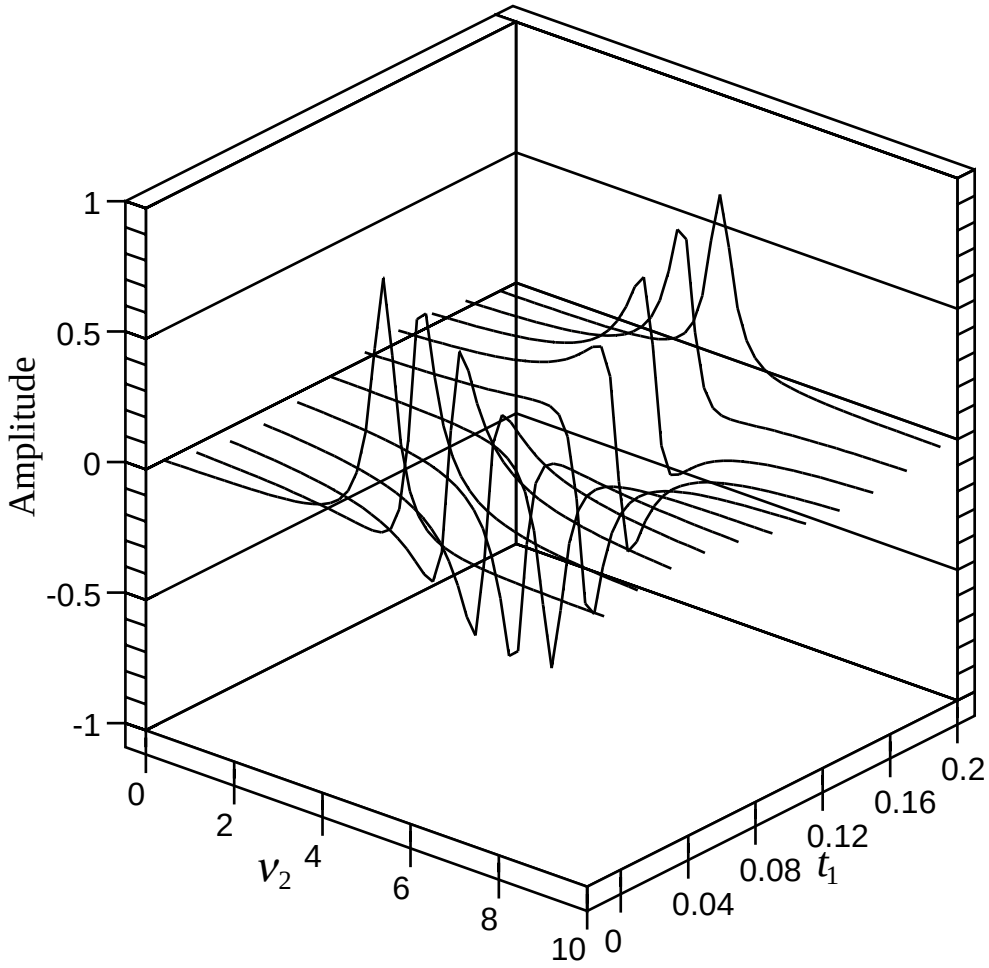


Figure 34

The signals to be cancelled out may be thousands of times larger than the desired signals or they may be much smaller.

14.4.1. Elimination of Detector Asymmetry.

One of the oldest applications of phase cycling eliminates the effects of unequal gain in the two quadrature channels of the spectrometer. The gains of the two channels in a spectrometer nearly always differ sufficiently to produce an observable image without phase cycling. The image appears as a result of foldover of the part of the signal in the higher gain channel which has no compensating signal in quadrature in the lower gain channel. Consider the consequences of altering the phase of successive pulses in an ordinary one-pulse FT experiment as shown in Figure 12 according to: 90_x, 90_y, 90_{-x}, 90_{-y}. Figure 36 shows the FID's obtained. Table I shows how the FID's can be combined to yield a final FID nominally the same as that resulting from four 90_x pulses. Note, however, that both the

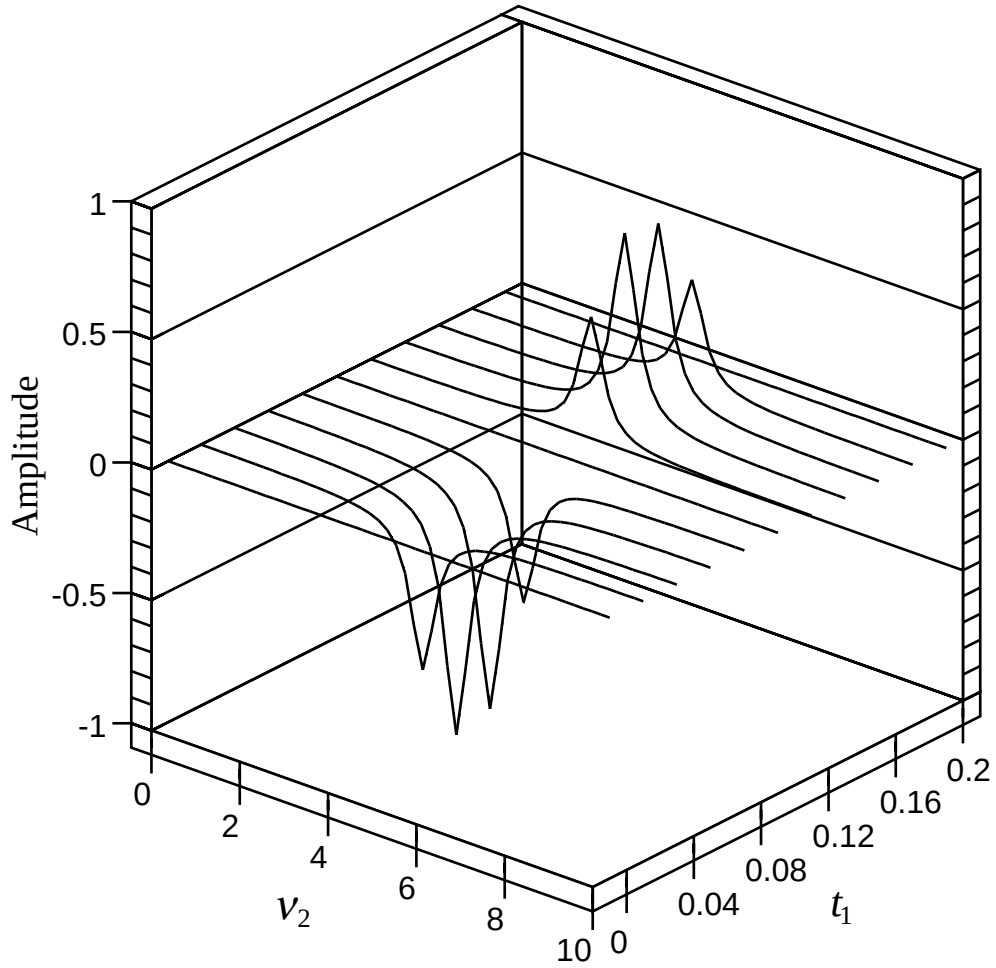


Figure 35

Table I: Pulse and receiver phases for quadrature image suppression.

	Receiver Phase	
Pulse phase	Real	Imaginary
+x	+y	+x
+y	-x	+y
-x	-y	-x
-y	+x	-y

real and the imaginary part of the summed FID uses each receiver channel in a symmetric way, so that any difference in gain between the two channels will be compensated exactly.

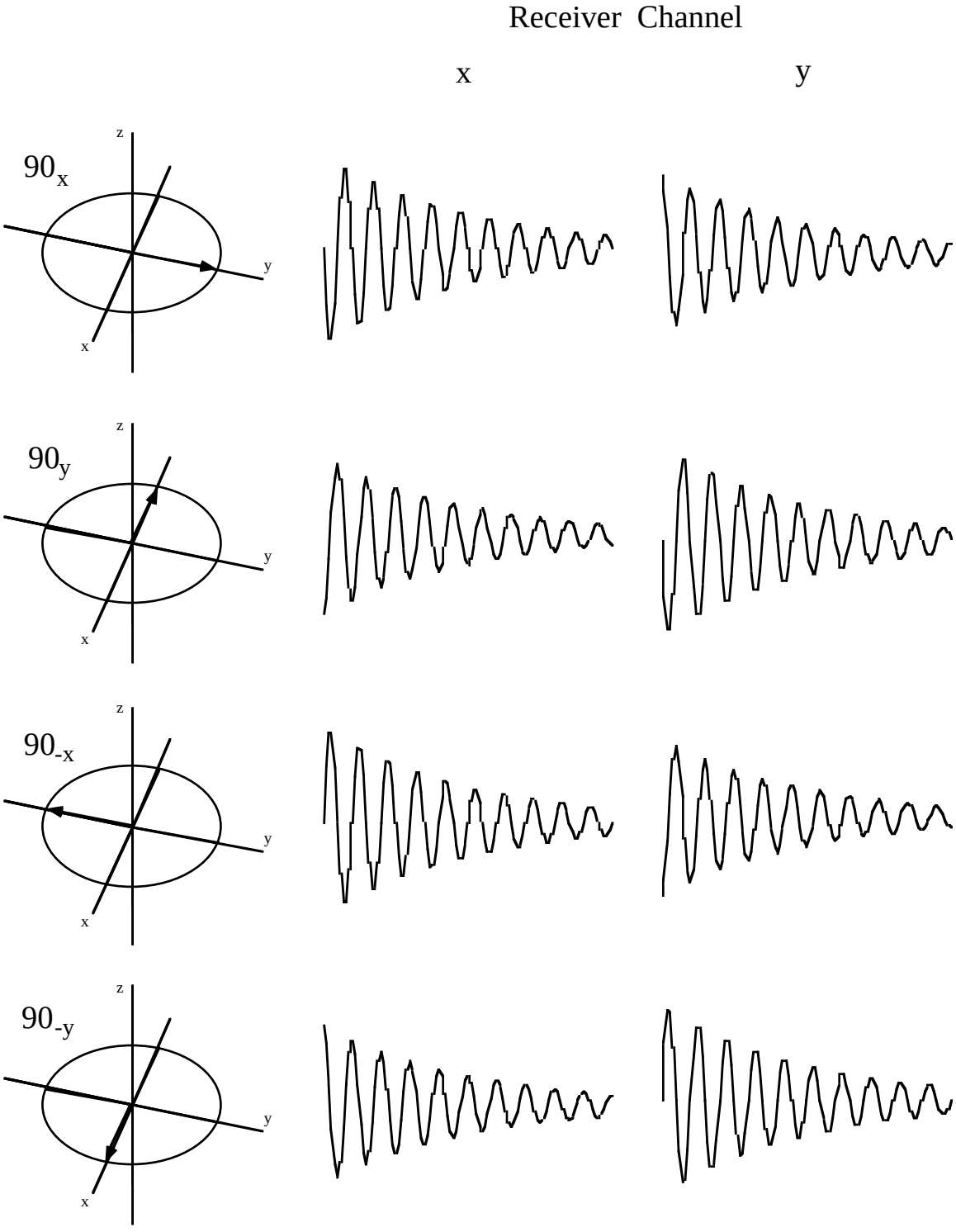


Figure 36

This example serves as an introduction to numerous other phase cycles used in pulsed NMR spectroscopy.

14.5. Pulsed Field Gradients.

Pulsed field gradients (PFG) can in many cases be used as an alternative to phase cycling. This is done by applying a pulse of current to a coil that causes a large inhomogeneity in the $\mathbf{B}_0$ field. It is as if, for example, the *z*-gradient of the shims were to be misadjusted for a short period of time, usually a few milliseconds. During this time, Spins at the center of the sample would not be affected and would precess at their usual Larmor frequency. But, as one moves higher in the sample, the spins would precess a little faster than at the center; and, as one moves lower, they would precess a little more slowly. Thus, the Larmor frequency would be a linear function of the z-coordinate of the particular spin. If one applies a ninety degree pulse followed immediately by a PFG, the transverse magnetization would seem to quickly disappear because the spins would dephase in the rotating frame due to their different Larmor frequencies. The magnetization as a function of z would look like a helix or cork screw. Now suppose we apply a PFG of opposite sign, i.e., the current flows in the opposite direction in the coil, for the same period of time. Now the spins above center in the sample would precess more slowly, while those below center would precess more rapidly. At the end of the second PFG the magnetization would have precessed back to its original phase and an echo would form. This is called a gradient recalled echo.

Now imagine we have a dilute solution of protein in water. We apply a ninety degree pulse followed by a PFG. All the magnetization dephases. Now we wait for a short period of time then apply the opposite PFG to recall the echo. The proton signals from the protein form the echo as expected, but the signal from the protons of water is much reduced. Why? The water molecules diffuse rapidly in the liquid compared to the large protein molecules. Thus, the water molecules at the end of the delay have, on average, moved to some other place in the sample with a different z-coordinate and do not correctly rephase, while the protein molecules do have the same z-coordinate as before and rephase just fine. By choosing the correct delay between the gradient pulses, the strong signal from water can be suppressed leaving the desired proton resonances of the protein.

Numerous other applications for pulsed field gradients have been published. This brief discussion serves only to give the flavor of what can be done.

15. Chemical Shift Correlation Experiments.

A large class of 2D experiments produces correlations between chemical shifts. The correlations occur as a consequence of some interaction between the nuclei whose chemical shifts are correlated. Various interactions, such as, scalar coupling, dipolar coupling, NOE, and chemical exchange, have been exploited by means of clever pulse sequences to yield structural and other information. Some examples will be discussed to illustrate how this can be done.

15.1. Homonuclear Shift Correlation (COSY).

The COSY experiment yields a 2D spectrum with both F_1 and F_2 labelled by the chemical shift of the nucleus observed (most often protons). Off diagonal peaks indicate scalar coupling between the two multiplets whose chemical shifts define the position of the peak. The peaks along the diagonal simply replicate the 1D spectrum. The pulse sequence used is shown in Figure 37. The COSY pulse sequence is deceptively simple; a full explanation of how it works is beyond the scope of this treatment; but some idea can be had by considering an AX spin system where both A and X are protons. The first 90 degree pulse creates transverse magnetization in both A and X which then evolves under scalar coupling as we have seen before in the heteronuclear case. The second 90 degree pulse transfers polarization from A to X and from X to A in somewhat the same way as the simultaneous pulses on protons and carbon did in the DEPT experiment. The magnetization precesses at the rate dictated by the chemical shift of the source nucleus during t_1 but that dictated by the destination nucleus during t_2. Thus, the correlation is produced.

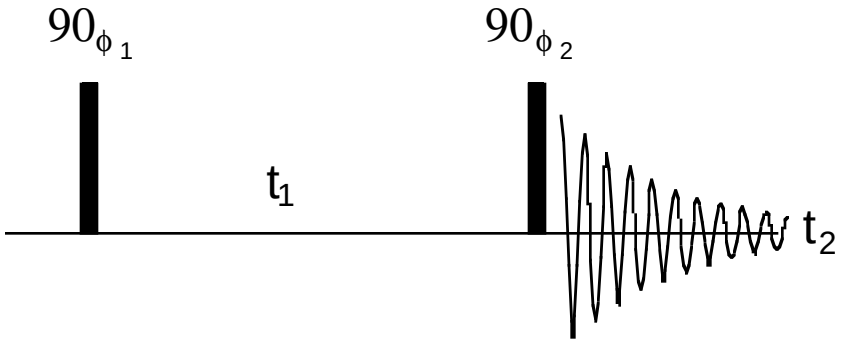


Figure 37

The phase cycling normally used is as follows:

$$\begin{aligned} \phi_1 &= (0,1,2,3)_4 \\ \phi_2 &= (0_4,1_4,2_4,3_4) \end{aligned} \tag{53}$$

The receiver follows the usual CYCLOPS sequence. The inner loop produces quadrature detection in the F_1 domain while the outer one suppresses quadrature images.

15.2. Carbon-Carbon Homonuclear Correlation, the 2D INADEQUATE Experiment.

The 2D version of the Incredible Natural Abundance Double Quantum Transfer Experiment (INADEQUATE), is one of the most elegant 2D structure elucidation techniques. A single spectrum has the potential to reveal the complete carbon skeleton of a molecule. The method depends on creation of double quantum coherence in a spin system consisting of two bonded ^{13}C nuclei. At natural abundance for ^{13}C (1.1%) about one molecule in ten thousand will have two ^{13}C nuclei participating in a particular bond. Thus, the method will be very insensitive compared to the more routine experiments observing carbon in molecules with only one ^{13}C nucleus.

The pulse sequence for this experiment is shown in Figure 38. The first three pulses constitute the preparation part of the sequence; they simply create double quantum coherence which is then allowed to evolve during t_1. Since no double quantum state is available for an isolated ^{13}C, the molecules with only one ^{13}C do not contribute to the double quantum coherences. The last 90 degree pulse brings the double quantum coherence back to single quantum antiphase magnetization which is detected as a function of t_2. The magnetization that was in the double quantum coherence during the evolution is amplitude modulated at the double quantum frequency as a function of t_1. Broadband proton decoupling is applied during the entire experiment, so proton-carbon couplings need not be considered. To get a satisfactory spectrum one must suppress the signals from the one hundred times more numerous molecules with only one ^{13}C. This is done by phase cycling as shown in Table II. The last 90 degree pulse (the read pulse) is cycled through all four quadrature phases. Since the single quantum and double quantum coherences respond differently, the receiver can be made to follow the double quantum coherences so that they add constructively while the single quantum coherences add destructively or cancel out. This experiment is normally performed using the phase sensitive hypercomplex method. This is

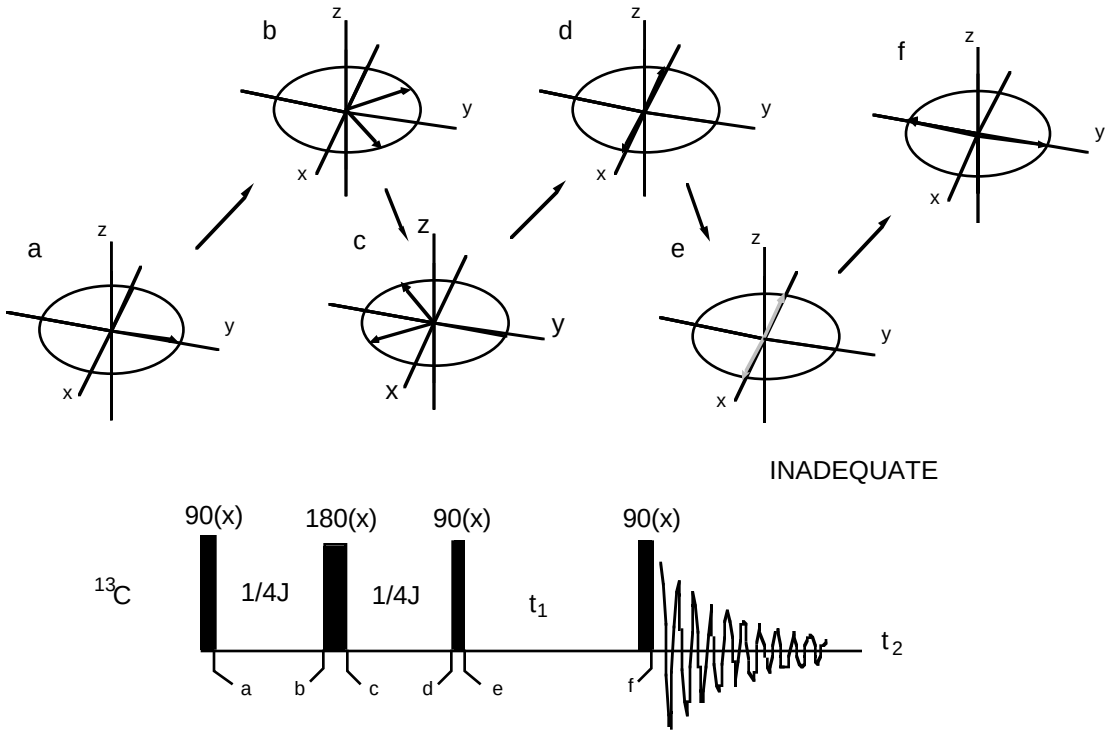
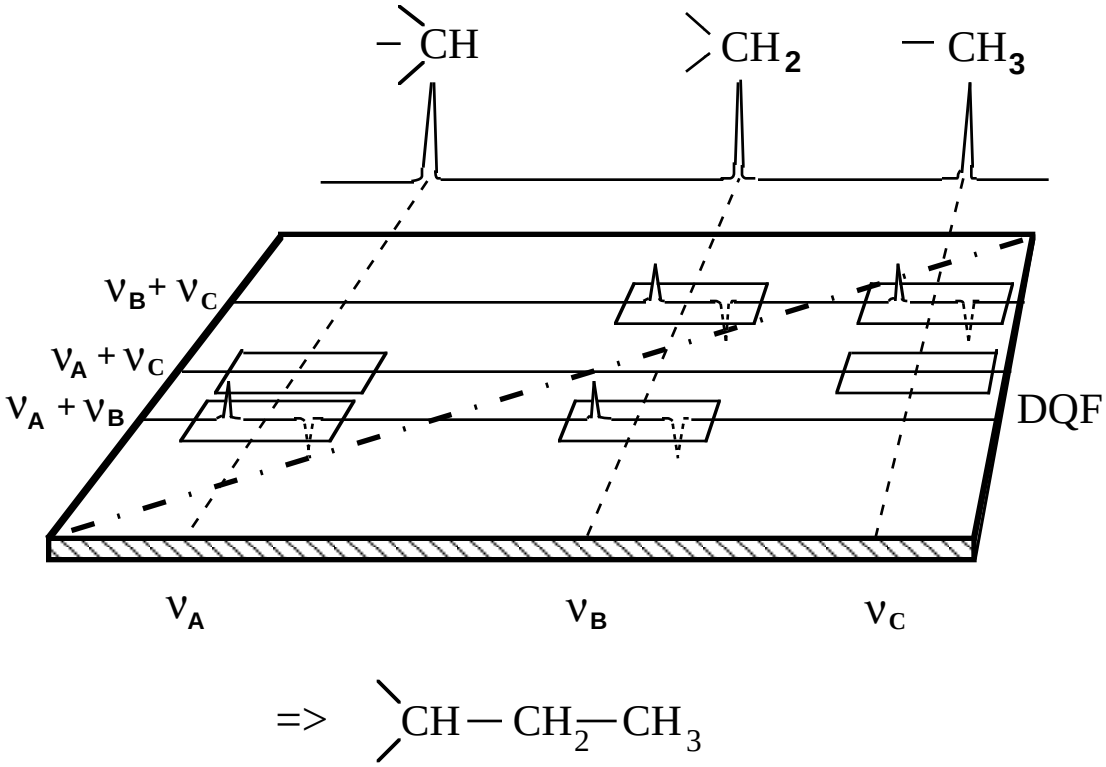


Figure 38

Table II: Basic Phase Cycle for 2D INADEQUATE.

Read Pulse	Single Quantum	Double Quantum	Receiver
+x	+x	+x	+x
+y	+y	-y	-y
-x	+x	-x	-x
-y	+y	+y	+y

accomplished by acquiring two data sets, identical except that one has the preparation pulses shifted in phase by 45 degrees. Special hardware to accomplish less than 90 degree phase shifts is required. Shifting the phase of the double quantum preparation by 45 degrees results in a phase shift of the modulation observed in the FID by 90 degrees as required by the hypercomplex method.



$$\Rightarrow \quad \begin{matrix} \diagup \\ \diagdown \end{matrix}\!\!\!

methyl (C) carbons also exists, but no bond pattern is found between the methine (A) and methyl (C) carbons.

15.3. Heteronuclear Chemical Shift Correlation.

This experiment produces a 2D spectrum with the F_2-axis labelled by the chemical shift of one nucleus (e.g., $\delta_{^{13}C}$) and the F_1-axis labelled by that of another nucleus (e.g., $\delta_{^1H}$). The coordinates of a peak in the spectrum would be ($\delta_{^{13}C}$, $\delta_{^1H}$), and existence of a peak at particular coordinates indicates scalar coupling between the relevant carbon-proton pair.

The pulse sequence and vector diagrams of Figure 40 illustrate how the experiment is accomplished. Since polarization transfer from ^{1}H to ^{13}C is involved, the carbon magnetization is enhanced as was described for the INEPT and DEPT experiments. Adjustment of the mixing delay sets the scalar coupling which will produce the maximum polarization transfer, hence, the maximum enhancement.

Phase cycling of the last proton pulse through all four quadrature phases will produce quadrature detection in F_1 and suppress signals not arising from polarization transfer from the protons.

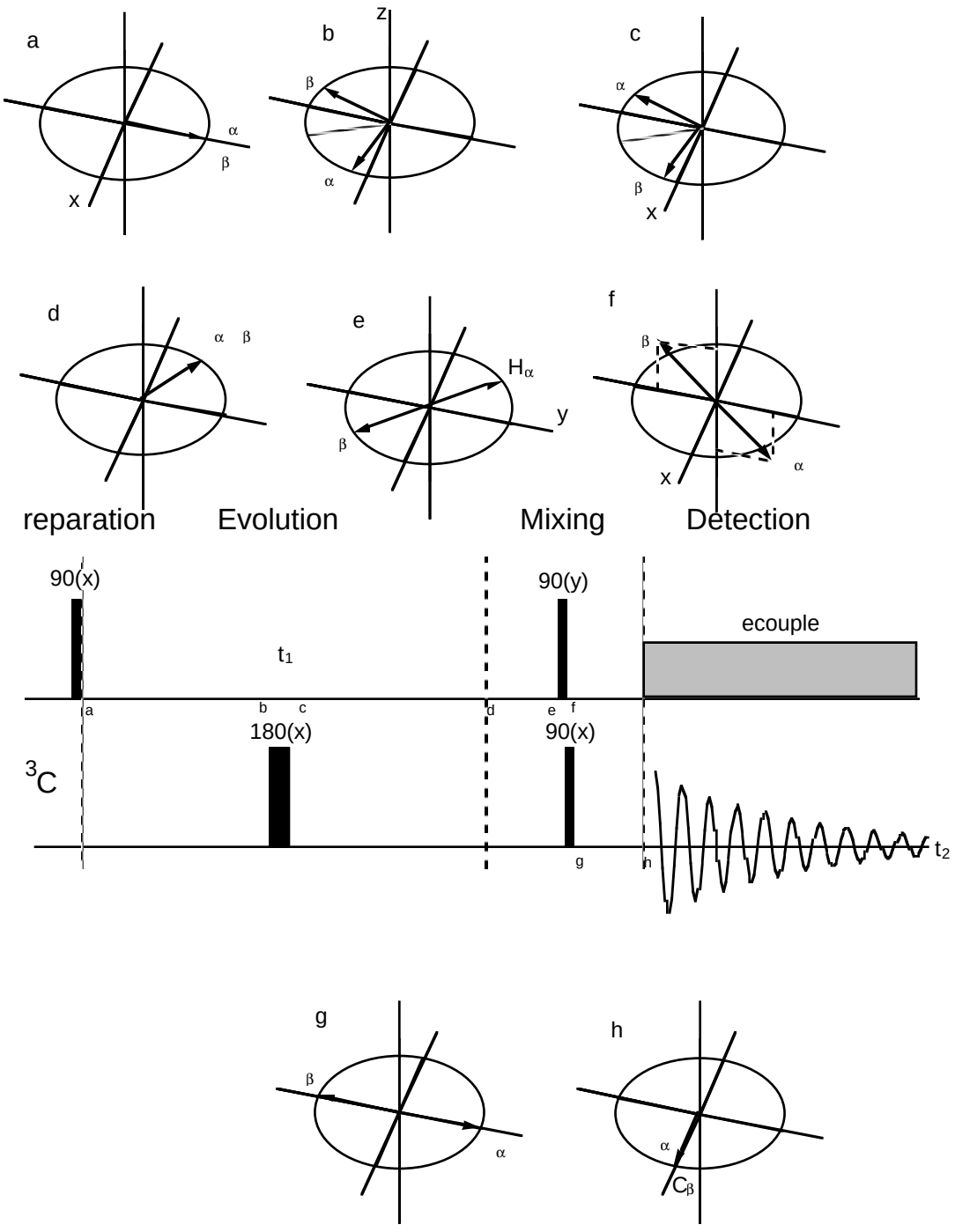

a
b
z
c
d
e
f
α
β
x
y
H_α
reparation
Evolution
Mixing
Detection
90(x)
t_1
90(y)
ecouple
180(x)
90(x)
^3C
t_2
g
h
C_β

Figure 40

16. Homonuclear and Heteronuclear J-Spectra.

Much of the complexity of a spectrum often arises from scalar coupling interactions between the nuclei involved. Overlapping multiplets can render interpretation difficult, and yet, the information contained in the scalar couplings is needed for a full structure determination. *J*-spectra permit one to separate chemical shift and scalar coupling information while retaining the full information content of both types. Furthermore, overlap between individual multiplets is usually eliminated.

16.1. Heteronuclear *J*-Spectra.

The classical case for heteronuclear *J*-spectra is the display of J_{CH} in F_1 while observing ^{13}C. Remember, however, that the technique can be used for other pairs of nuclei as well. There are two ways to do this experiment. The gated decoupler method is the simplest to implement but leads to scaling of the coupling constants by a factor of 1/2. Figure 41 shows the pulse sequence for this method.

> Problem 29: The other method for heteronuclear *J*-spectroscopy leaves the decoupler off during all of t_1 and places a 180 degree proton pulse simultaneous with the 180 degree carbon pulse. Make a diagram of this pulse sequence similar to Figure 41 and explain how it works. Why are the coupling constants not scaled by a factor of 1/2?

16.2. Homonuclear *J*-Spectra.

Figure 42 shows the pulse sequence for homonuclear *J*-spectroscopy.

> Problem 30: Add vector diagrams to Figure 42 showing how the magnetization evolves for an AX spin system. Show the effects of chemical shift and scalar coupling. Discuss how the sequence works. What is refocussed and what is not refocussed?

Since there is no analog of broadband decoupling in a homonuclear system, the scalar couplings appear in both domains. Hence, the multiplets appear along a line tilted at 45 degrees. If the rectangular data matrix is distorted into a parallelogram with angles of 45

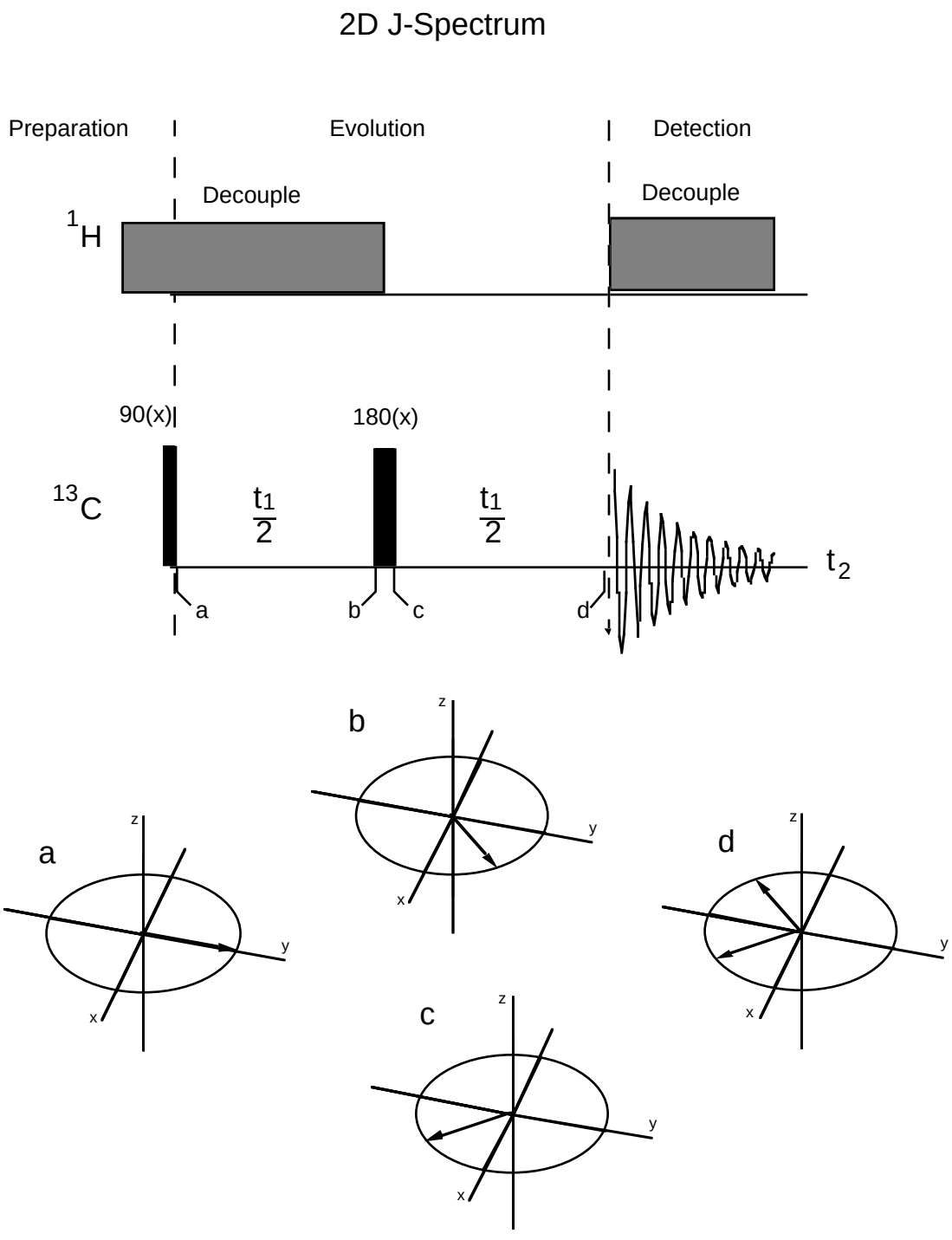

2D J-Spectrum
Preparation
Evolution
Detection
Decouple
Decouple
1H
90(x)
180(x)
13C
t1/2
t1/2
t2
a
b
c
d
z
y
x

Figure 41

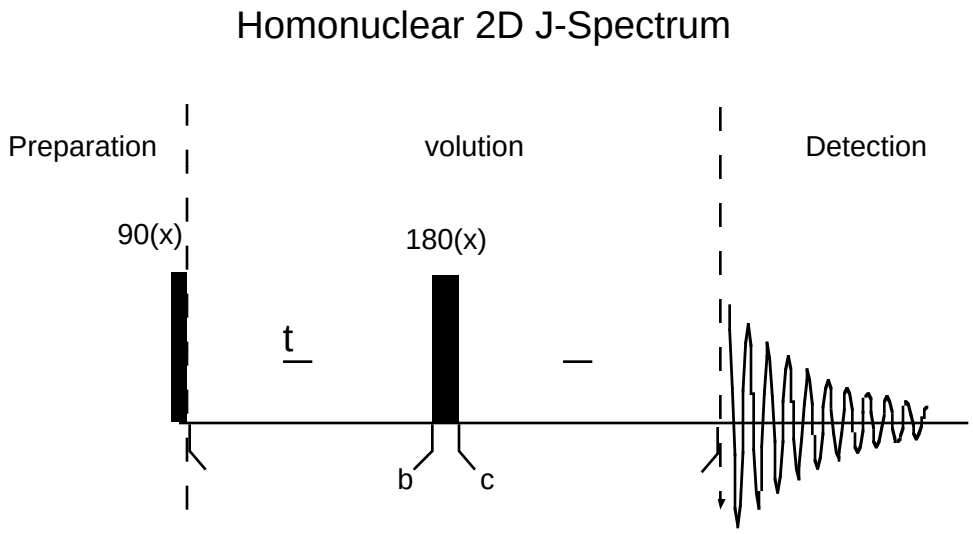


Figure 42

and 135 degrees, the data will appear nearly as for the heteronuclear case. A projection on F_2 will then produce, effectively, a broadband decoupled proton spectrum.

17. A Basic Pulsed Fourier Transform Spectrometer.

A pulse and Fourier transform spectrometer must include hardware and software components to implement all of the parameters and concepts which we have, so far, discussed in a fairly theoretical framework. Of course, the physical reality never perfectly matches the theoretical model, so it is important to understand where the spectrometer falls short of our theoretical models and what effect this has on our data. In this section the various parts of the spectrometer will be discussed with the intent of showing how the experiments that have been discussed are actually carried out

17.1. Generating $\mathbf{B}_0$—The Magnet.

17.1.1. Types of Magnets

17.1.1.1. Iron Core Electromagnets.

17.1.1.2. Permanent Magnets.

17.1.1.3. Superconducting Solenoids

17.1.2. Field Homogeneity Adjustment—the Shim System.

17.2. Generating $\mathbf{B}_1$—the observe transmitter.

17.2.1. Crystal Controlled Oscillator.

17.2.2. Frequency Synthesizer.

17.2.3. Phase Shifters.

17.2.4. Gating Circuits.

17.2.5. Attenuators.

17.2.6. Power Amplifiers.

17.2.6.1. Linear vs. Tuned Amplifiers.

17.2.6.2. Pulse vs. Continuous Amplifiers.

17.3. Detecting the Nuclear Induction Signals—the Receiver.

17.3.1. Preamplifiers.

17.3.2. Mixers.

17.3.3. Analog Filters.

17.4. The Data Processing and Spectrometer Control System—the Computer

17.5. Maintaining a Constant Field/Frequency Ratio—the Lock System.

17.6. Generating $\mathbf{B}_2$—the Decoupler.

17.7. Controlling the Sample Temperature—the Variable Temperature System.

17.8. Coupling All the Other Subsystems to the Sample—the Probe.

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Part II
Laboratory Experiments

Introduction.

This section contains a series of laboratory exercises designed to introduce you to various aspects of high resolution pulse and Fourier transform NMR. The experiments are designed to illustrate the principles presented in the accompanying lecture series. They are designed around a Varian Inova 500 NMR spectrometer, although any spectrometer running the vnmr software and configured with the required probes and hardware options may be used. A switchable broadband probe is sufficient for all the experiments. Various other probes, such as, broadband, indirect detection, four nucleus, or proton/fluorine may be substituted for performing individual experiments. This will be discussed in greater detail in each experiment.

This laboratory manual is not intended to replace the Varian User's Manuals. All the manuals are available on-line. Paper manuals are no longer distributed in quantity by Varian. The paper manuals found in the spectrometer rooms generally pertain to older versions of the software, but the on-line manuals will always be the proper versions for the installed software. Pertinent sections of these manuals may be consulted prior to each session on the instrument if you are unfamiliar with commands and parameters used. Vnmr and the manuals are accessible from many workstations and personal computers in the department. These may also be used for examining your data and preparing reports after your sessions on the spectrometer. Hard copy may be sent to several alternative printers. Check with one of the NMR staff if you don’t know a convenient place to get access.

These experiments presume that the student has completed the basic instrument checkout, and has a basic knowledge of how to interpret one-dimensional proton and carbon-13 spectra. Contact the analytical instrument manager to arrange for training and checkout if this has not already been completed.

It is best to perform the experiments in sequence if one is inexperienced with NMR techniques because each successive experiment presumes familiarity with the techniques used in preceding laboratory sessions. Also, the directions for the experiments become less explicit as one progresses through the course, under the presumption that the student is becoming familiar with various procedures and does not need explicit instructions. Once the techniques have been mastered, the various experiments may be used as guides for analyzing other samples.

Laboratory 1: Proton Line shape, Proton Resolution.

Before beginning this laboratory you should have completed the basic checkout. This laboratory will serve as a review of that checkout and expand upon the skills gained in the checkout training. In this laboratory particular aspects of the spectrometer such as safety procedures to protect vulnerable components, basic disk operations such as rebooting the system software, accounting and log in procedures, location of the various components that make up an NMR spectrometer, and basic operations such as locking, probe installation and tuning, and shimming the magnet will be reviewed.

You should become very familiar with these procedures so that you can verify that the spectrometer is correctly adjusted and working well before starting any long experiment. Each time you log in on the spectrometer workstation a checklist will pop up on the screen. Verify that each item is satisfied before attempting to run the spectrometer. These instruments are used heavily by the department research groups; and, because of the large number of users, strict protocols will be required of you. During this and subsequent sessions, spectrometer time (magnet hours) will of necessity be limited. Whenever possible practice and data analysis should be done on workstations other than those attached to the spectrometers. Spectrometer hours should be used mainly for data acquisition.Consult your instructor for the locations of these workstations.

Only standard Varian commands and macros are used in this exercise. You should become familiar with these and not rely on the convenience features defined by the Department NMR staff until you are comfortable with the standard commands. The reason for this is to familiarize you with the system as supplied by Varian. Any Varian instrument running vnmr that you may encounter will have this same set of commands and parameters.

1.1. Demonstration.

The following items will be discussed and demonstrated by the instructor.

a. System protocols: scheduling, accounting, logs, good citizenship.

b. Safety procedures, spectrometer components, and host workstation operation.

c. Safety in handling samples and chemicals in the NMR laboratories.

d. What to do when the system doesn’t respond normally.

e. Sample: Sample preparation, tube quality, sample tube positioning. (**Read** Appendix 2: on page 141.)

f. Probes: Probe observe and decoupler channel tuning, range sticks, probe filter and cable arrangements.

g. Locking and shimming: Finding a deuterium lock signal manual and computer shimming, loading standard shims. (**Read Appendix 1: on page 137.**)

h. Data acquisition: starting, stopping, monitoring an acquisition while in progress. For this experiment acquisition parameters will be given to you. Later you will learn how to create and modify the parameters to suit your purposes.

i. Phasing and plotting: use of manual and automatic phase adjustment, use of remote printers and plotters.

j. Saving data and transferring it to permanent archival storage. (See "Chemistry NMR Laboratory, Application Note No. 1, Data Archiving." Copies are available in the spectrometer rooms and the document can be viewed on the web at:

http://www.chem.utah.edu/chemistry/facilities/nmr/app1.html.

1.2. Pertinent Parameters and Commands.

The following parameters and commands are frequently used. Most of them may be accessed from the menu buttons as well as by command line entry. Study any that you are not familiar with in the User's Manual prior to doing this experiment. **acqi, axis, cexp, delexp, rt, rts, nl, dres, res, delta, dsn, aph, svf, lockgain, lockpower, lockphase, go, ga, au, file, solvent, load, copy, tn, dg, dg1, dgs, explib, ft, wt, wft, wti, text, su, ds, dscale, jexp, sa, ra, pap, ppa, pl, pscale, page**. In most cases there are menu buttons to access the same functions as these commands. You may use them if you prefer. A mix of both methods will be used in the following procedure.

1.3. Setting Up, Shimming, the Line Shape Test.

In this exercise you will preform all of the operations that were demonstrated above. These operations are basic to all NMR experiments. Here you will create an experiment, set up the required parameters by loading a predetermined set (Determining reasonable parameters for data acquisition will be the topic of future sessions.), optimize magnet homogeneity, obtain an FID, process it, and store the results. The line shape test is the most important test in setting up the spectrometer to observe protons. If good results are obtained for the chloroform line shape, other samples usually present few problems.

1.3.1. PROCEDURE:

a. Log in on the paper log book, and on the spectrometer workstation. Complete the on-screen checklist.

b. Insert the proton lineshape sample.

Open the **acqi** window by typing the command or by clicking the **acqi** button in the menu bar. Click the **eject** button to raise the sample. Be sure to handle the spinner turbine with a tissue. Skin oil from your hands will accumulate and cause the sample not to spin. The lineshape sample is chloroform in acetone–d_6. Before inserting the sample in the spinner turbine, be sure that the sample tube and spinner turbine are clean. Before releasing the sample into the upper barrel be sure there is sufficient air to support the sample at the top of the barrel, then click the **insert** button to lower the sample into the probe. Click **close** to close the acqi window.

c. **explib**.

This command will display a list of the experiments currently existing in your home directory. You have your own copy of the experiment files that cannot be changed by other users. The experiments will be just as you left them when you last logged out. Notice the size of the experiments. Do not leave unneeded experiments (especially large ones) in your home directory. Archive the data you want to keep and delete the experiment using **delexp**.

d. **cexp**(n).

where n can be 1 through 9 but must not be the same as for one of the already existing experiments. This creates a new experiment file where you can perform data acquisition without disturbing whatever is in the other experiments. Caution! exp5 is used by some commands as buffer space for the results. It is best to avoid this one until you are very familiar with the software.

e. **jexpn**.

Join experiment n that you just created.

f. Click the following buttons in sequence: **Main Menu**, **File**, **Set Directory**, **More**, **VNMR**. Now click on stdtests in the file list; it will be highlighted; then click **Change**. Click the file name corresponding to the probe you are using and click **Change**. Click last_h1lshp.fid/ in the file list, then **Return**, then **Load**.

This retrieves the last line shape test run by one of the NMR staff. If you wish, you can type **wft** to see what the line shape looked like at that time. Using this technique you can navigate to and load any fid file into the current experiment.

g. load='y' su.

When a fid file is saved, all the parameters values in effect at that time, including all the shim values, are saved with it. The spectrometer has two computers. One is called the “host” and is used for handling the screen, keyboard, and peripherals as well as doing computations such as Fourier transforms; this is the Sun workstation you are now using. The other is called the “acquisition” computer and is located inside the spectrometer console. This computer actually controls the spectrometer making the pulses and delays, digitizing the FID etc. **su** tells the host to transfer the acquisition parameters that you have entered to the acquisition computer. Among other things it sends the appropriate pulse sequence code and checks which nuclei will be observed and decoupled and sends the appropriate frequencies to the acquisition processor. The frequencies correctly reflect the offsets you have entered. The values entered for **tn**, **dn**, **tof**, and **dof** determine the observe frequency and the decoupler frequency. This is why the probe cannot be correctly tuned until a **su** command has been executed; the probe does not necessarily receive the correct frequencies until this command is completed. The **load** parameter controls whether or not the values of the shims are also sent to the acquisition computer; all other acquisition parameters are always transferred. In this case, since we have set **load** to ‘y’, the values of the shims are transferred and loaded into the shim DAC’s. Whenever you type a **go**, **ga**, or **au** the spectrometer will automatically precede the acquisition with a **su** to recognize any changes you may have made to the acquisition parameters. The **su** command automatically sets load to ‘n’ after it completes the transfer. It is advisable to always keep **load** set to ‘n’ unless you are specifically loading standard shims. Otherwise you may accidentally enter an experiment containing nonoptimal shims and transfer these to the shim DAC’s replacing the set you have carefully optimized.

h. Click **acqi** in the menu bar, and then click **lock** in the acqi window.

Lock the spectrometer to the acetone in the sample as you were shown in the demo.

i. Tune the probe Be gentle; the probes are fragile and expensive (more than $20,000 each); if they have to be returned to the factory for repair, they are usually gone for several weeks.

j. **ga dres**.

Collect a spectrum showing the line shape prior to any shimming and display it on the screen. Place a cursor on the line and use **nl** to put it precisely on the line. Note that **nl** gives the height of the line in millimeters. **dres** then displays the width of the peak at half height. The width of the peak at any other height may be measured using two cursors and the threshold. Display the line at two vertical

scales one to have the entire peak observable on the screen (**vs** = 100, **wp** = 25 Hz, the best **vs** value may vary depending on the plotter you are using) and another with **vs** increased by a factor of one hundred with **wp** = 250. Use normalized mode, **nm**. Learn how to increase and decrease the vertical scale using the mouse and how to save and recall views of the spectrum using the **sn** and **rn** commands. The main chloroform resonance will be far off scale under the hundredfold expansion. Phase the spectrum using **aph**. Also click the **phase** button in the menu bar and learn to manually phase the spectrum. The line width at 0.55% of full intensity is measured as follows: Display the hundredfold expanded spectrum with an appropriate horizontal expansion and set the horizontal threshold bar (click the **th** button in the menu bar) at 0.55% of the full height of the line. Remember, you get the line height using **nl**. Using the left mouse button to control the position of the cursor pair and the right mouse button to control the spacing between the cursors, place a cursor on each side of the peak where it intersects the threshold bar. The value of **delta** displayed at the bottom of the screen gives the width of the line. If **delta** is in ppm, it may be changed to hertz by setting **axis**='h'. The value of **delta** in hertz may also be obtained by typing **delta**? on the command line. Note that the current value of any parameter may be obtained this way**.** Make another measurement with the threshold set at 0.11% of the line height. Once the magnet is properly shimmed the three values obtained should closely match those of the spectrum from which you took the starting shims, and the line shape should be symmetrical, devoid of "chairs" resulting from incorrect even order Z-gradients. If you spend the time to get proper line shape, on the chloroform sample, it is usually easy to get adequate resolution on all other samples. If you cannot get good chloroform line shape, you should not expect to get good results on other samples.

k. pl pscale pap page.

You can do this step and all the subsequent plotting for this experiment later on one of the data processing workstations if you wish.

Make a hardcopy for the 50%, 0.55%, and 0.11% measurements. Use the **text** command to label the spectra appropriately. Be sure to include in the text the line widths measured at various heights, your name, the name of the spectrometer you used, and any other notes you want to include about the spectrum. In this case, for example, you probably want to note that the spectrum was taken before any shimming. The backslash, \, character starts a new line when the text is printed. Type **text** with no arguments when you want to see how the label will look when printed. This text will be saved with the data and will be printed with the parameter listings when you execute **pap.**

It is also possible, and probably more convenient, to use any standard Unix text editor to create this text file. If time permits, ask your instructor to show you how.

l. Save your spectra in your archive directory. Your instructor will show you how.

m. Now you are ready to practice shimming. Enter the interactive acquisition window just as you did when locking the spectrometer. Shim the magnet as well as you can. Adjust only the Z1, Z2, Z3, and Z4 shims. You will not be required to adjust the other shims at this time. Accidentally misadjusting them can cause increased spinning sidebands or worse. Alternately shim the magnet and monitor the spectrum by typing **ga** until you get a satisfactory line shape. Your instructor will judge whether your spectrum is good enough to continue. Monitor the line shape as you did with the unshimmed spectrum above. If the standard shims you loaded are recent enough, it may be quite easy to meet specifications. Even if this is the case, you should explore the effect of the various shims on the line shape. Next time you might not be so lucky.

n. When your best spectrum has been collected, add text, make hard copy, and archive the data as you did above.

o. Many newer spectrometers can produce good line shape even without sample spinning. This is often desirable for running 2D and other more complex experiments. Turn off the spinning. Now adjust the **x1** and **y1** shims for maximum lock level. Acquire, label, plot, and archive another spectrum. This will allow you to judge if this spectrometer can be used with nonspinning samples later on.

p. at=1.5

This changes the acquisition time to 1.5 seconds. What effect do you think this will have on the spectrum of chloroform?

q. ga

Collect another spectrum with the new acquisition time. Note any change in line shape. Document the three line shape parameters as you did above. Make plots and archive the data. No matter how well shimmed the magnet is you cannot obtain the best resolution unless you have adequate acquisition time to produce a well digitized line.

r. All these hard copies should be included and discussed in your report for this laboratory.

s. Make a permanent archive of your data on CDR. Your instructor will help you if needed.

t. Shut down the spectrometer and log out observing all the protocols you were taught during the demonstration.

Laboratory 2: Observation of Carbon-13.

In this session you will learn how to switch from observing protons to observing carbon-13, determine a signal to noise ratio, do a 90 and 180 degree pulse width calibration, and determine a ^{13}C T_1 relaxation time.

2.1. New Commands and Parameters.

In addition to the ones you learned in the first laboratory exercise, the following commands and parameters should be studied for this laboratory class: **tn**, **pw**, **p1**, **array**, **da**, **dssh**, **dssn**, **ds**(*n*), **sw**, **at**, **np**, **fn**, **lb**, **movetof**, **movesw**, **d1**, **d2**, **ernst**, **ll**, **dll**, **dot1**, **t1**(n), **expl**, **dps**.

2.2. Observation of an X Nucleus.

The design and construction of probes is an exercise in compromise. No one probe can be constructed that is optimal for all experiments that one might wish to perform. Thus, one must consider whether a given experiment is optimal, or even possible, with a certain probe.

In NMR terminology an X nucleus includes all NMR active nuclei other than protons (and usually ^{19}F). Most broadband probes will observe nuclei with gyromagnetic ratios in the range from ^{15}N to ^{31}P. Tuning the probe to the desired frequency is accomplished by changing the capacitance or inductance of resonant circuits. Coarse tuning is normally done by inserting an appropriate range stick, which is a fixed capacitor or inductor attached to a plastic rod, into the probe. Fine tuning is then done by adjusting variable capacitors attached to adjusting rods protruding from the bottom of the probe. Various probes have slightly different arrangements for doing this. There are two main types of probes: Broadband probes are designed such that the X observe coil is located closest to the sample for maximum sensitivity, and the proton decoupler coil is located further away since proton sensitivity is not as much an issue for decoupling. Indirect detection or HX probes are constructed with the proton coil close to the sample for best proton sensitivity and the X coil further away. The probes are redundant in that the HX probe can be used to observe an X nucleus and decouple ^{1}H or the broadband probe used to observe ^{1}H and decouple X; but, in both cases, signal to noise and resolution will not be optimal.

2.2.1. PROCEDURE:

a. Log in and perform the checklist.

b. Check with your instructor to make sure the correct probe is installed.

c. Insert and lock the carbon-13 sensitivity sample. Do not use the doped sensitivity sample, the purple one.

This sample contains 40% dioxane - 60% benzene-d_6.

d. Tune the probe observe channel and decoupler channel. Your instructor will teach you how to do this for the particular probe you are using. Mishandling of the probe tuning adjustments can damage the probe. If, you are not perfectly sure of what you are doing, get help.

e. Retrieve the latest carbon lineshape test for the probe you are using from the stdtests directory, load the shims and parameters from this file, then lock and shim as you did in Laboratory 1.

f. Check the carbon-13 lineshape at 50, 0.55, and 0.11% of full height as you learned to do for proton lineshape in Laboratory 1. Shim the magnet as needed to achieve performance similar to that in the file you retrieved from stdtests. Add appropriate text and save the FID in the archive for use in preparing your report.

g. You have been using decoupled dioxane (**dm**='y') to check the lineshape. Now let's see what happens when the decoupler is turned off by setting (**dm**='n') and acquiring another spectrum. Remember that the decoupler doesn't actually turn off until after the **su** command is executed.

The dioxane peak should be a 1:2:1 triplet. Be sure to wait at least one minute between successive acquisitions to allow for full relaxation. You can increase **d1** if you want to ensure this, but usually the time to process the data will be sufficient. Note the fine structure in each of the dioxane triplet resonances. This is due to long range coupling to the six protons not directly bonded to the carbon-13. Save this FID for your report also. Note the difference in S/N between the coupled and decoupled spectra.

h. Retrieve the latest signal-to-noise test for this probe. Transform this data and measure the S/N using the **dsn** command. Now acquire a spectrum and check that the S/N is close to that of the standard data set. Your results should be within 5% of the standard.

2.3. Calibration of Observe Channel Pulse Widths.

The extent to which the magnetization is perturbed from thermal equilibrium is determined by the transmitter power and length of time the transmitter is left on. (See the lecture notes Section 5. on page 12 for more detail.) Our objective is to determine the length of the pulse necessary to produce a 90 or a 180 degree pulse at a given transmitter power. This is done by observing the amplitude of a strong signal near the carrier as a function of

pw; the amplitude will trace out one full cycle of a sine function between zero and 360 degrees. The pulse widths corresponding to the positive maximum and the first zero crossing will be the 90 and 180 degree points respectively. Due to a small off-resonance effect and to $\mathbf{B}_1$ inhomogeneity, the 180 degree zero crossing will not be a perfect null but will appear as a dispersion mode signal with much reduced amplitude compared to the 90 degree point. Calibration of the 90 and 180 degree pulses is required for 2D NMR pulse sequences, T_1 and T_2 determinations, and determining optimal values for **pw** when rapid pulsing is required. The pulse width calibration for a particular nucleus will vary depending upon the type of sample, and will be different from one nucleus to another even using the same probe. Samples that are highly ionic are likely to cause longer than normal 90 degree pulses. If the pulse width calibration is critical for a particular experiment, it is best determined on the sample to be analyzed or on a similar sample, and not on a standard sample.

2.3.1. PROCEDURE.

a. The doped carbon-13 sensitivity sample will be used for this calibration. It is exactly like the regular carbon-13 sensitivity sample, except that it has been doped with a paramagnetic relaxation agent to shorten the relaxation time. It is easily recognized by the purple tint imparted by the relaxation reagent.

b. Duplicate the parameter set you used for the S/N experiment into some other experiment using the **mp** command.

c. Join the experiment where you just put the parameters using the **jexp** command.

d. Turn on the decoupler and set the decoupling parameters as you did for the lineshape test above.

e. Set the text to something appropriate.

f. **ga**

This will collect an initial test FID. Transform this FID and then use the **movetof** command to set the transmitter frequency within about 200 Hz of the dioxane peak. Take another spectrum to see that you have succeeded. The dioxane peak should be near the center of the spectrum. The purpose of this step is to ensure that off-resonance effects will not cause problems with the 180 degree pulse calibration. (See Figure 7 and the accompanying discussion.)

g. Use absolute intensity, ai, so that peak heights from spectrum to spectrum can be compared with accurate relative intensity relationships. The usual display mode is normalized, **nm,** which scales the tallest peak in the spectrum to the value of **vs** in millimeters. Center the dioxane peak as you did before in the S/N test and adjust **vs** so

the peak is about 1/3 full screen in height. Adjust the **phase** so as to have a pure absorption signal then DO NOT adjust the phase in subsequent steps.

h. **d1= 5**

This is a relaxation delay of 5 seconds which allows for the magnetization to return to thermal equilibrium before the next pulse. The calibration will not be accurate if **d1** is set too small.

i. Check the standard spectrum book to see what the nominal width of the 90 degree pulse should be. Type **array** and answer the questions so as to have **pw** arrayed with about 50 values between 0 and 450 degrees. The array values will be displayed when the command completes. The array may be redisplayed by typing **da** anytime. Check to see that the array is correct. Individual values in the array may be changed or elements added to the array as follows, for example: **pw**[51]=45. Try this to add one more value to your **pw** array.

j. **ga**

Start the acquisition. The amplitudes of the dioxane peak should trace out a little more than one full cycle of a sine curve. If the curve is not smooth or not sinusoidal, this is usually an indication of probe arcing. Try again with a lower value of **tpwr**.

k. You can monitor the acquisition in progress using the **dssh** and **ds**(*n*) commands where *n* is the spectrum number assigned to a particular **pw** array element when you display the array. Adjust the vertical scale (**vs**) and vertical position (**vp**) as needed so that all the spectra are on scale. If the first few spectra indicate that the range of **pw** is not correct for the value of **tpwr** you are using, abort the acquisition and readjust the array so as to obtain a little more than one full cycle of the sine wave.

l. Archive the data.

m. Now reset your parameters so that the dioxane line is about 10,000 Hz off resonance. Repeat the pulse width calibration to see what effect being off resonance has. Archive this data. Include in your report diagrams to show where $\mathbf{B}_{eff}$ is in the two cases and how this influences your pulse width calibration.

n. When you make hard copy for your laboratory report, try both pap and ppa to add a parameter list to your spectrum and note the differences.

2.4. Determination of a Carbon T_1.

The method used to determine T_1 in this session will be inversion recovery. The pulse sequence is shown in Figure 22 of the lecture notes. The delay, **d1**, allows the mag-

netization to come to thermal equilibrium. The 180 degree pulse, **p1**, then places the magnetization along the negative z-axis (assuming no off-resonance or $\mathbf{B}_1$ inhomogeneity effects). The magnetization relaxes toward thermal equilibrium due to T_1 (spin lattice or longitudinal relaxation) processes for some period of time, t_1 controlled by the parameter **d2** which will be an arrayed parameter to permit monitoring of the full relaxation curve. The amplitude of the partially relaxed magnetization is then measured by application of a 90 degree pulse, **pw**, to tip the magnetization into the *xy* plane, where it will produce an FID as a function of t_2 during the acquisition time, **at**. A critical aspect of this experiment is allowing enough time between the end of the acquisition and the next 180 degree pulse (**d1**) for complete re-establishment of the thermal equilibrium magnetization to occur. Since the T_1 relaxation is exponential, 5 T_1's are needed to recover about 99% of the thermal equilibrium magnetization. *A priori* you do not know how long this delay must be since this is what you are determining in a T_1 experiment; you must, therefore, put in a best guess. This relaxation delay may need to be only a few milliseconds (for quadrupolar nuclei) or many minutes (for a highly isolated spin-1/2 nucleus). It is difficult to generalize, but a guess for a T_1 for protons in small organic molecules would be 1-10 seconds while carbons would range from 1-100 seconds. You should also realize that, if there is more than one nuclear spin in a molecule, and this is almost always the case, relaxation can be nonexponential. For proton decoupled ^{13}C spectra relaxation is almost always close to exponential, but in coupled spin systems it need not be.

Problem 31: Draw and explain vector diagrams describing the inversion recovery T_1 sequence.

For quick approximations, since $T_1 = T_2$ for nonviscous isotropic liquids, the relation

$$T_1 = T_2 = \frac{1}{\pi L} \tag{54}$$

where L is the full width of the line at half maximum in Hz, can be used to set a lower limit on T_1. Since many other factors, such as, magnet inhomogeneity and unresolved scalar coupling, can broaden lines; it is only a lower limit and can be in error by orders of magnitude in some cases.

The intensity of the line as a function of t_1 is given by

$$M(t) = M(\infty) + \left[M(0) - M(\infty)\right]\exp\left(-\frac{t_1}{T_1}\right) \tag{55}$$

As **d2** (i.e., t_1) is increased from a value much smaller than T_1 to a value much greater than T_1, the intensity of a line in the spectrum will start out negative pass through zero and asymptotically approach a maximum value. The value of **d2** at which the intensity is zero, let us call it t_{null}, is related to T_1 by

$$T_1 = \frac{t_{null}}{\ln 2} = \frac{t_{null}}{0.693} \tag{56}$$

This gives a much better approximation to T_1 than the line width, but is still fairly crude.

Problem 32: Derive Equation (56).

T_1 is more accurately determined by plotting ln[$M(\infty)$-$M(t_1)$] vs. t_1. $M(\infty)$ is the amplitude corresponding to the maximum signal, i.e., t_1 is infinite, and $M(t_1)$ is the signal amplitude at each value of t_1. The slope of the line gives the value of $1/T_1$.

Problem 33: Show that the slope of the log plot does give T_1.

The most accurate way to calculate T_1 is to do a three parameter nonlinear regression using T_1, $M(0)$, and $M(\infty)$ as adjustable parameters. This is the way the spectrometer software does it. We will not go into the details of the algorithm here.

Experimentally, T_1 is dependent upon several factors, these include temperature, solvent viscosity, and the presence of paramagnetic impurities. Note that molecular oxygen, O_2, is paramagnetic; therefore, dissolved oxygen can decrease the measured value of T_1. This may be undesirable in cases where one is trying to accurately measure dipolar relaxation or NOE. See Section 6. on page 15 and Section 12. on page 45 for additional details.

2.4.1. PROCEDURE.

a. Eject the 40% Dioxane sample and insert the sample supplied by you instructor for this laboratory session.

b. Lock, tune the probe, shim the magnet, and obtain a decoupled spectrum

Tune the probe and shim the magnet as before. Obtain a spectrum using **nt=1** and a large spectral width (**sw =** 50000) at first then narrow the spectral width using the **movesw** command so as to include just the peaks from the compound and about 500 Hz of signal free region on each end of the spectrum.

c. **np**=16384

Change the number of data points to 16384 and note the automatic change in acquisition time (**at**).

Problem 34: Explain why **at** changed.

d. **dp=’y’; fn**=32768 **dm =** ‘y’

e. **pw90=***x*

Enter *x*, the value for the 90 degree pulse you determined in part 2. When the spectrometer sets up the T_1 experiment it will use this value to set **pw** to a 90 degree pulse and **p1** to a 180 degree pulse.

f. **ai**

Problem 35: Why must one use absolute intensity in this experiment?

g. **dot1**

This is a macro to set up a T_1 experiment. The macro will look at the value in **pw90**, copy it to **pw**, double it, and enter it as a value for **p1,** then will determine what value to use for the equilibration delay, **d1,** and the values to put in the **d2** array, by asking you what values are expected for the minimum and maximum T_1's and how long you want the experiment to last. Use the following: Minimum = 0.1 second, Maximum = 5 seconds, and a total experiment time of 0.5 hours.

h. **da**

This will display the array for **d2** that the macro determined for you.

Problem 36: Explain why the values in the array are reasonable choices based on your answers to the questions in the **dot1** macro.

i. **dg**

Note the value for the relaxation delay **d1** calculated by the macro.

Problem 37: Explain why this value is a reasonable choice.

j. **dps**

This will display the pulse sequence showing the pulses and delays. It should look similar to Figure 22. This command can be used anytime to verify that the pulse sequence variables are set up correctly. Parameters other than the default pulses and delays can be viewed. See the command and parameter reference manual for details.

k. Add appropriate text for this experiment.

l. ga

Start the acquisition. As the acquisition proceeds monitor the acquisition with dssh. Also experiment with a stacked presentation using the **dss** command and appropriate values of **ho** and **vo** to produce a satisfactory display.

m. ds(*n*)

Display the last spectrum in the array. (Do a **da** if you have forgotten how many values were used for **d2.**) Phase the spectrum.

Set the threshold **th** so it is just below the top of the smallest peak in your spectrum. The threshold menu button is probably most convenient for this. Note the changed assignments of the mouse buttons when you press this menu button.

n. dll

This will display a line listing of your spectrum. The list will give (for each peak that is above the threshold limit you set) a reference number, an intensity, a frequency, and a chemical shift. In this session T_1's for all the lines will be calculated, but, in some cases, only a few may be of interest. When this is the case the line numbers assigned to the peaks of interest are used in the **t1(***n***)** calculation in the next step.

Problem 38: Learn how to reference the spectrum to TMS using the center peak of the chloroform triplet as a secondary reference. Check the **rl** command in the manual.

o. fp t1

The **fp** command examines every spectrum in the array to determine the peak intensities for each peak picked in the previous step. **t1** calculates the T_1's for all the lines in the line list. When only a few of the lines in a spectrum are of interest the **t1** command can be given an argument containing a list of the line numbers of interest obtained from a line list as above. Details of the fitting process and the resulting T_1 values are displayed in the text window. If full detail is not needed, the **t1s** command gives only a brief summary of the calculated values.

Anything sent to the vnmr text window can alternatively be sent to the printer by issuing the **printon** command. When it is desired to resume sending text to the text window on the screen issue the **printoff** command. Text is only queued for printing until the **printoff** command is completed, then all the accumulated text is sent to the printer.

p. expl

This will display the best fit exponential curves calculated by the program as well as your data points on the screen. This can be used to spot bad data points. The command **pexpl** will send the display to the plotter instead. This is a very versatile command; check the manual for details.

Problem 39: Make a log plot of the T_1 data for any <u>two</u> lines in the spectrum and determine the value of T_1. Make a table comparing your result with the T_1 values calculated from measuring the linewidths, by estimating the zero crossing, and with the value calculated by the **t1** command.

q. Archive your data as before.

r. Be sure to use all the data analysis techniques you have learned in preparing your laboratory report.

Problem 40: Suppose you have a proton spectrum with two lines in it. Each line represents one proton in your molecule. One line has a T_1 of 10 seconds, the other 1 second. A spectrum is run with an acquisition time of 1 second and a **D1** of 4 seconds Based on what you have learned in this experiment, what will happen to the intensities of these lines? What will happen when you integrate the spectrum and try to count protons?

Now suppose the T_1's of the two protons are equal with one of them resonating at 5 ppm, which is nearly on resonance. The second proton is at 10 ppm, which is far enough off resonance so that $\mathbf{B}_{eff}$ differs appreciably from $\mathbf{B}_1$. If one collects a spectrum using a 90 degree pulse calibrated correctly for the 5 ppm proton, will the integrals of the two resonances be equal? Why or why not? (Hint: you may want to review Section 5.2.)

Discuss how one would need to set up the spectrometer to ensure that accurate integrals would be obtained in a proton spectrum if $\mathbf{B}_1$ is not adjustable? Hint: Consider what happens for pulses much less than 90 degrees.

Laboratory 3: Decoupling and the NOE.

In this laboratory exercise some of the effects obtainable with the use of the decoupler will be demonstrated. It should be noted that 2D experiments exist which give the same information in one experiment as would be obtainable from many decoupling experiments of the type described here. These 2D experiments are often replacing the 1D counterparts now, even for routine analyses. However, it is useful to understand the principles involved in the 1D experiments; and, sometimes, when a single piece of information is all that is required, the 1D experiment is preferable.

3.1. New Commands and Parameters.

In addition to the ones you have already learned the following new commands and parameters will be used in this experiment: **rts, gamah2, h2cal, dof, dm, dmm, dmf, dlp, sd, sda, homo, int, li, dli, ins, axis, rl, rfp, split**.

3.2. Homonuclear Single Frequency Decoupling.

Single frequency decoupling, as its name implies, involves irradiation of the sample with a coherent single frequency at the chemical shift of one of the nuclear species in the sample. Since it is a second irradiation in addition to $\mathbf{B}_1$, it is called $\mathbf{B}_2$. When the $\mathbf{B}_2$ power is much greater than the scalar couplings, the scalar couplings are effectively removed from the spectrum. Consequently, these scalar couplings disappear from the multiplet pattern of any nuclei that are scalar coupling partners of the one being irradiated, thus, identifying the partners. The term homonuclear means that both coupling partners are of the same nuclear species, e.g., ^{1}H{^{1}H} (This is the one we will do.), ^{19}F{^{19}F}, ^{31}P{^{31}P} etc. The heteronuclear case will be dealt with in the next section. This technique may be used to track down the origin of a coupling present in a multiplet or to simplify a multiplet pattern to determine the value of a particular scalar coupling.

3.2.1. PROCEDURE:

a. Log on to the system and perform the checklist.

a. Check with your instructor to be sure the correct probe is installed.

b. Insert sample labeled PHENETOLE, set up a proton experiment, tune the probe, lock and shim the magnet starting from the best proton line shape shims you have obtained previously. The **rts** command may prove useful.

c. Run a preliminary spectrum with a sweep width sufficient to insure

that no lines are aliased. Be sure the **solvent** parameter is set correctly. **np** should be at least 32 K points. (**fn** should then be 32 K or larger, or set **fn = 'n'**.). Set **dn** and **tn** to protons.

d. Decrease the spectral width to a more appropriate value, and take another spectrum. Be sure you are adequately digitizing the FID. **movesw** and **df** should be useful.

e. Reference your spectrum to TMS using the center line of the solvent multiplet as a secondary standard. **nl** and **rl** should be useful.

f. Expand the region containing the triplet and the quartet of the phenetole and make a hard copy for later comparison with the decoupled spectrum. Be sure to display a scale in ppm under the spectrum and print the parameters. You may want to save this display using **s1**. Be sure to archive the data in case you want it again later. Learn how to create a subdirectory in your archive and place all the data from this experiment in it.

g. Expand the region containing the quartet until you can place a cursor accurately at the center of the multiplet. The **split** command may be useful.

h. Use **sd** to set the decoupler frequency to the cursor position.

Write down the value of **dof**; you will use this offset later for heteronuclear single frequency decoupling.

i. homo = 'y'

We want to do homonuclear decoupling. Setting the decoupler modulation mode, **dmm**, to **c** is not operationally necessary but should be done to remind you that you are doing cw decoupling.

j. dpwr=28

k. Run a preliminary spectrum then increase or decrease the value of **dpwr** as needed to the lowest power that will totally collapse the high field triplet. Make an array of **dof** over a range of 10 Hz around your original value and observe the effect. Try 2 Hz steps to begin then 0.2 Hz steps in the vicinity of the best value. Was your original value the optimum one? Be sure to save the data for your report.

l. Make a hard copy of the best decoupled spectrum just like the one you made for the coupled spectrum.

m. If time permits, repeat the above procedure irradiating the triplet.

3.3. Heteronuclear Single Frequency Decoupling.

The principles involved in heteronuclear decoupling are precisely the same as in homonuclear decoupling. The difference is that the observed isotope will be different from

the decoupled one. Some common combinations are $^{13}C\{^{1}H\}$, $^{31}P\{^{1}H\}$, $^{15}N\{^{1}H\}$, $^{1}H\{^{31}P\}$

Problem 41: Why would a $^{1}H\{^{13}C\}$ or $^{1}H\{^{15}N\}$ experiment be of marginal utility at natural abundance? (Hint: think about the isotope distribution in the molecules. How many different isotopimers will there be, and what will each one contribute to the proton spectrum.) There are 2D experiments which can overcome this limitation. What is the most important property such a 2D experiment would have to have?

3.3.1. PROCEDURE:

a. Use the PHENETOLE sample as above; tune the probe to ^{13}C; and obtain a broadband proton decoupled spectrum. If you need to, you can discuss with your instructor reasonable values for **sw**, **at**, **np**, **d1**, **pw**, **dn**, **dof**, **dm**, **dmm**, **fn**, and **lb**. Use at least 64 K data points. Obtain the current correct values for **dmf** and **dpwr** for the instrument you are using from the standard spectrum book for your spectrometer.

b. Reference your spectrum to TMS using the solvent line, display a scale in ppm, add appropriate text, and archive the data.

c. dmm=c dof=*x*

where x is the decoupler offset that you made note of when you were doing homonuclear decoupling. This will change the decoupler modulation mode from waltz modulation (**w**) to coherent single frequency (**c**).

d. Rerun the spectrum with the new decoupler parameters and enough transients to obtain good signal to noise. You will probably need to decrease the decoupler power to avoid perturbing the other couplings, but it should be obvious immediately which carbon is bonded to the quartet protons.

e. Add text and archive the data as usual.

Problem 42: How might this experiment be useful in solving research problems? Give hypothetical examples for your own research if you can.

3.4. Gated Decoupling and the NOE.

The phenomenology behind the NOE is discussed in the lecture notes, Section 11. on page 41 and will not be repeated in detail here. Suffice it to say that a $^{13}C\{^{1}H\}$ NOE

depends on the number of protons and their distances from a given carbon atom and on the nature of the relaxation mechanisms involved. The value can range from one (no enhancement) to about three. This is the principal reason why carbon spectra run with broadband decoupling, as is normally done, should not be integrated with the intention of counting carbon atoms the way one normally counts protons. (Off resonance effects and relaxation effects can also influence the intensities.) Integration of a ^{13}C spectrum may be preformed on data that was acquired with the decoupler off, but overlapping multiplets usually make this a rather unsatisfactory alternative. However, by running the decoupler gated in such a way as to produce a decoupled spectrum with no NOE referred to as a "suppressed NOE" experiment one can circumvent this difficulty. Other methods such as the addition of "shiftless" relaxation agents such as chromium(III) acetylacetonate $Cr(acac)_3$ have been used to produce integrable carbon spectra but will not be described here.

The suppressed NOE experiment consists of gating the decoupler off during a delay period before the observe pulse then switching it on during the acquisition. Decoupling happens immediately, whereas, the NOE builds up at a rate controlled by T_1, and, thus does not affect the amplitude of the resonances.

The "opposite" experiment to that described above is a coupled spectrum with NOE. This type of spectrum is obtained by allowing the NOE to build up with the decoupler on during some time period before the observe pulse then switching it off during the acquisition time. Coupling information is present in the FID as soon as the decoupler is turned off, but the intensities of the resonances are those characteristic of the NOE enhanced spectrum. This is often the method of choice to obtain coupled carbon spectra because of the increased signal-to-noise afforded by the NOE.

3.4.1. PROCEDURE:

a. Use the PHENETOLE sample as before. Set up and obtain a broadband decoupled ^{13}C spectrum. Now set **nt=16**, **d1=30 sec** and use **ai.** Create a decoupler mode array by typing

   dm='yyy','nny','nnn','yyn'. Acquire the four spectra.

b. The rest of this experiment can be done at a workstation if you wish. Learning to use the integration tools may be a bit time consuming.

c. Integrate the methyl and methylene regions in the first two spectra. Learn to use the menu buttons to control the integration and establish integral resets. The **dc** (drift correction) and **bc** (baseline correction) commands can be used as needed, to help the signal free regions to integrate to zero, as they should. Be sure the spectrum is

correctly phased before applying these corrections.

d. Display the second spectrum expanded to show the integral of the methylene and methyl carbons. Set **ins**=1 and place the cursor within the integral region of the methylene. Use the macro, **setint**, to normalize this integral to one. Use **dli** to list the integrals. With the NOE suppressed, the integral of the methyl should also be one within experimental error. Now display the first spectrum and note the integrals of the methyl and methylene. The values of these integrals are your measured NOE values for these two carbons.

e. Learn to use the various display and plot commands for integrals, such as: **dli**, **pir**, **pirn, setint**.

f. Determine the signal-to-noise ratio for the methyl and methylene multiplets using spectra three and four.

Problem 43: Explain why your integrals of the first spectrum obtained above give the values of the NOE for the methyl and methylene carbons. Is the increase in signal to noise from the third to the fourth spectrum consistent with your measured NOE? Discuss how the various experiments might be useful in solving research problems? Give examples relevant to your own research if you can.

Laboratory 4: Observation of a Less Common X Nucleus

In this session you will have the opportunity to observe a nucleus other than the common ones (protons, fluorine, carbon, and phosphorus) that are NMR active. Experiments involving these four do comprise the bulk of present day NMR data; nevertheless, data from the numerous other NMR active nuclei can often be useful. You will be given a choice of which nucleus you wish to observe from the list given below. The list is not exhaustive but has been selected based upon natural abundances, and relative sensitivities to be practical to complete in one laboratory period without isotopic enrichment.

Most elements occur naturally in more than one isotopic form. The fraction of a natural sample of an element that is a given isotope is termed the natural abundance of that isotope. The natural abundance of some isotopes varies substantially depending on the source of the sample. Only isotopes which have a nonzero nuclear spin can be detected by NMR. The natural abundances of NMR active isotopes vary widely. For some elements, such as, hydrogen, fluorine, and phosphorus the natural abundance of one NMR active isotope is essentially 100%. For example, the natural abundance of ^{1}H is about 99.98% the remainder being ^{2}H. For carbon, on the other hand, the natural abundance of the isotope that is NMR active, ^{13}C, is only 1.1%, while the rest is essentially all ^{12}C which is NMR inactive. The element iridium has two natural isotopes ^{191}Ir and ^{193}Ir the former has an abundance of 37.3% while the later is 62.7%. Each has a spin of 3/2, both are NMR active but each isotope has a different gyromagnetic ratio, γ, and, hence, resonates at a different frequency. Even many low abundance quadrupolar nuclei, such as ^{2}H, can be observed fairly easily with modern spectrometers.

Relative receptivity or sensitivity gives you an idea of how strong a signal you might expect to get from a particular nucleus. The values are generally given relative to either protons or carbon-13. For nuclei of natural abundances, N_r for the reference nucleus and N_x for the nucleus of interest, the relative receptivity, D_r, is defined as

$$D_r = \frac{N_x}{N_r}\left|\frac{\gamma_x^3 I_x(I_x+1)}{\gamma_r^3 I_r(I_r+1)}\right| \tag{57}$$

where *x* refers to the nucleus of interest and *r* refers to the reference nucleus. The absolute value is used so that D_r will always be positive in spite of the negative γ of some nuclei. Relative sensitivities for some of the common nuclei with respect to protons are given in Table I.

Although many nuclei have low natural abundance and low sensitivities, time averaging allows collection of many of these on a routine basis. Sometimes low sensitivity can be circumvented by using pulse sequences that are able to take advantage of the scalar coupling of an insensitive nucleus to a more sensitive nucleus, such as ^{1}H. This process is referred to as polarization transfer and is the basis for several 1D and 2D NMR experiments.

Problem 44: Independent of relative sensitivity considerations, relaxation effects can have a great influence on the ease with which spectra can be taken of insensitive nuclei. Discuss how T_1 and T_2 might affect your ability to get a spectrum of such a nucleus (a) if T_1 and T_2 are both very long, (b) if T_1 is very long and T_2 is very short, and (c) if T_1 and T_2 are both very short. Note that T_1 cannot be very short and T_2 very long because this would imply that the magnetization recovers to thermal equilibrium along the *z*-axis before the *xy* magnetization has relaxed to zero yielding a net magnetization greater than M_0, which is impossible.

4.1. New Commands and Parameters.

The following commands and parameters should be studied for this experiment in addition to the ones you have already learned: **tn, dn, spcfrq, movetof**.

4.2. Outside preparation.

ALL OF THE FOLLOWING SECTION MUST BE COMPLETED BEFORE YOU ARRIVE FOR YOUR INSTRUMENT SESSION.

Table I: Relative receptivity of some common isotopes.

isotope	R_H
^{19}F	0.83
^{31}P	$6.6 \cdot 10^{-2}$
^{11}B	0.13
^{17}O	$1.08 \cdot 10^{-5}$
^{15}N	$3.8 \cdot 10^{-6}$

From Table II choose one nucleus you wish to attempt to observe in this session. Note that the entry for protons is given only for reference and is not to be chosen. You may choose a nucleus that is relevant to your research or have your instructor help you choose an interesting one. Use the Handbook of High Resolution Multinuclear NMR to find the basic information about your chosen nucleus. Pay particular attention to natural abundance, chemical shift range, chemical referencing methods, and relaxation times. Ask your instructor where to find the handbook.

With your instructor, choose one or two compounds containing the nucleus of interest and make up appropriate samples. One sample should contain a strong easily observed signal, if possible, of known chemical shift so that you can reference your spectra.

Table II: Natural Abundance and Larmor frequency of some selected isotopes.

Nucleus	Spin	% Natural Abundance	Larmor Frequency (MHz)
^{1}H	1/2	99.98	100.0
^{7}Li	3/2	92.6	38.864
^{11}B	3/2	80.4	32.084
^{15}N	1/2	0.37	10.133
^{17}O	5/2	0.037	13.557
^{19}F	1/2	100	94.007
^{23}Na	3/2	100	26.451
^{27}Al	5/2	100	26.057
^{29}Si	1/2	4.7	19.865
^{55}Mn	5/2	100	24.664
^{77}Se	1/2	7.58	19.067
^{79}Br	3/2	50.54	25.053
^{111}Cd	1/2	12.75	21.205
^{117}Sn	1/2	7.61	35.625
^{195}Pt	1/2	33.8	21.499

If your first sample contains only a single resonance, you may want to make a second sample using some more interesting compound.

4.3. General Considerations.

Nominally correct frequencies for most isotopes are coded into the vnmr software. The tables containing these data are located in /vnmr/nuctables. These are just ASCII files. You can take a look at them with an editor, if you wish, to see which nuclei are included. Based on the type of spectrometer you are using, vnmr will choose the correct file from the nuctables directory. Then, setting **tn** will get you in the correct frequency range for your nucleus. If the chemical shift dispersion of your chosen nucleus is large, wide sweep widths may have to be used. (The maximum allowable sweep width on the Inova spectrometers is 500 kHz; on the older models it is generally 100 kHz.) However, you need to consider the spectral width you can reasonably expect to cover with the $\mathbf{B}_1$ field strength you have available. (Refer to Section 5.2. on page 13.) You may need to use more than one value of **tof** if a very wide range of chemical shifts needs to be checked. When a signal is found, move the spectral window to put your signal(s) in the center of the spectrum and narrow the spectral width to include just the signals of interest. Increase the acquisition time to adequately digitize the FID if necessary. Verify that the signals are not aliased and are true NMR signals, not glitches. Glitches are spikes that can occur in the spectrum and are from electronic sources, not from nuclei. They usually are only one data point wide, no matter how long the acquisition time is, and have random phase. Because of the random phase, they will usually become less prominent with time averaging. They may come in clusters that look a little like multiplets. The spectrometer manufacturers generally try to exclude glitches from the regions where commonly observed nuclei resonate, but it is impossible to exclude them from all regions where less commonly observed nuclei are found. Be aware that line broadening and other convolution functions can cause glitches to appear to be more than one data point wide. With weak signals you may need to time average to see a convincing resonance. When you have enough signal-to-noise you should be able to see an exponentially decaying FID using **df**.

4.4. PROCEDURE:

a. **Do not attempt this step without assistance, unless you have been trained to install probes.** Install a probe appropriate for the nucleus you have chosen. Check that the instrument is operating properly by running either a carbon-13 or proton lineshape test as appropriate for the probe.

b. Calculate the approximate Larmor frequency for the nucleus you

have chosen. Set **tn** and verify that the spectrometer frequency is close to the one you calculated. Set up reasonable parameters for acquisition considering the properties of the nucleus you will observe. Remember to execute an **su** before attempting to tune the probe. Install the correct quarter wave cable on the preamp for the nucleus you will be observing. If needed, install the correct fixed capacitor or inductor stick in the probe, then tune.

c. Search for a signal considering all the hints that were given in the previous section.

d. Narrow the spectral width if necessary and acquire a well digitized spectrum of reasonable signal-to-noise.

e. Reference your spectrum to a known chemical shift, if possible. If not, explain why not.

f. If time allows, run a 90 degree pulse width calibration and T_1. Does this information suggest any changes in the parameters you used above?

g. Your discussion of this experiment should include as many aspects of the particular nucleus as possible, for example, chemical shift and referencing, coupling constants, relaxation characteristics, etc. Be sure to discuss how these characteristics influence optimum choice of spectrometer operating parameters. Did you make appropriate choices for the spectra you obtained? How would you do things differently if you need to observe this nucleus in the future? If the spectrum you obtained has more than one resonance, explain their origins and determine the values of any coupling constants. (Learn how to use **dll** and to print the line list. Also, learn to use **dpf** and **ppf**.)

Laboratory 5: Distortionless Enhancement by Polarization Transfer (DEPT)

The observation of many nuclei is hindered by both low natural abundance and low gyromagnetic ratio leading to poor sensitivity and, consequently, to severe limitations on the problems to which NMR spectroscopy of these nuclei can be applied. When such an insensitive nucleus is coupled to a nucleus with a higher gyromagnetic ratio, e.g., ^{1}H, by a dipole-dipole interaction, enhancement of the insensitive nucleus can be achieved by taking advantage of the NOE, as has been shown in a previous laboratory exercise. A different method which relies on the scalar coupling between the sensitive and insensitive nuclei can be used to transfer the high degree of polarization of the sensitive nucleus to the insensitive nucleus. The DEPT experiment is one of a large family of one and two dimensional experiments that make use of this polarization transfer technique.

The DEPT and the closely related INEPT experiments result in signal enhancements on the order of the ratio of the γ of the sensitive nucleus to that of the less sensitive nucleus. The latter method, however, results not only in polarization transfer via scalar coupling, hence a signal enhancement, but also produces multiplet intensity ratios which do not conform to those characteristic of thermal equilibrium. In fact, the sum of the intensities of all the lines of a multiplet will be zero; whereas the DEPT sequence produces signals that are positive with the usual intensity patterns, hence, the name “Distortionless”. Characteristic of both methods is the fact that, unlike the NOE, the enhancement is independent of the sign of γ. The pulse repetition rate is governed by the T_1 of the sensitive nucleus (which is usually shorter than that of the observed nucleus), hence, one can use shorter relaxation delays with a resultant decrease in the total experiment time. The most common source of polarization is ^{1}H, but nuclei such as ^{19}F and ^{31}P are also used. To be useful for this purpose a nucleus must have high natural abundance and a large value of γ.

The INEPT and DEPT pulse sequences were invented as tools for enhancement of insensitive nuclei, but they are currently far more commonly used for differentiating between CH, CH_2, and CH_3 moieties in a molecule. Consult the lecture notes, section 13. on page 48, for details of the pulse sequence and how the signal intensities for the various CH_n moieties depend on the width of the final proton pulse. This dependence can also be used to calibrate the decoupler pulse. This calibration will be useful for 2D experiments which you will perform later.

In this laboratory session you will learn three ways to find out about various aspects of spectrometer operation: (1) on-line manuals, (2) the **dps** command, and (3) pulse sequence source code. Study the use of each of these carefully, as they will be very valuable in subsequent sessions and in your later NMR work.

5.0.1. PROCEDURE:

a. Join or create an experiment. Insert the ^{13}C sensitivity sample, set up for ^{13}C observe, and check the lineshape. Shim as necessary.

b. Insert the sample provided by your instructor and obtain a carbon spectrum with broadband proton decoupling. Use a 90 degree observe pulse width, **nt** = 1, and 32 K data points, set the decoupler offset to zero making sure the value of **solvent** is correct. This will place the center of the broadband proton decoupling near 5 ppm. If your proton chemical shifts are known to be localized in some narrow region of proton chemical shifts, the decoupler offset can be modified appropriately. Remember that the frequency axis increases from right to left not left to right as usual. Thus, if you wanted to set the decoupler offset to 3 ppm on a 500 MHz spectrometer, for example, you would set **dof** to -1000 Hz. Narrow the sweep width so as to include only the region of interest and the solvent signal. Check the standards book or the stdtests directory for pulse widths and power levels if you do not have them for the instrument and probe you are using. Save this spectrum for future reference.

c. You may want to try this step on a workstation prior to your spectrometer session. A set of manuals identical to the paper manuals exists on-line. Learn how to use them by looking up the DEPT experiment as follows:

   Click the right mouse button anywhere in the background window. A menu will pop up; click “On-line Manuals” with the left mouse button. If the appropriate menu item is not present you can start the on-line manual reader by typing “vnmr_ihelp” at any unix prompt. Once the reader displays the list of available manuals, select “*VNMR Command and Parameter Reference*” then click “D” in the alphabet list at the left side of the display. Select “dept” in the list at the left side of the window; you may need to scroll the list. This brings up a brief description of the dept macro. Among other things you will note an item that says “See also, “*User Guide: Liquids NMR*”. Since there is no hypertext link you have to go back to the main menu by clicking the “to menu” item in list at the left side of the window.

   This takes you back to the menu where you can select “*User Guide: Liquids*”. The list at the left of the window is a hypertext table of contents for this manual; click on the small triangle to the left of “Index”. Select “D” in the sublist that opens up, then find “dept” in the

main window which now displays the relevant section of the index. You may have to scroll to find the correct entry. Click on the hypertext page number to go to the corresponding page in the manual. Your browser is now pointed at the section of the manual relating to the DEPT experiment. Study these pages to learn more about how to run DEPT on the Varian spectrometer. You can iconify this window by clicking the "dot" button in the upper right corner of the window and bring it back to refer to it by double clicking on the icon.

This general procedure can be used to look up anything in the manuals.

d. **printon dg printoff**.

This prints a hard copy of the **dg** window for reference.

e. dept

This is the macro you just looked up in the on-line manuals which loads in the proper pulse sequence and modifies or creates other pertinent parameters for the DEPT experiment. The macro assumes that you have already set up to run a normal carbon spectrum, so be sure the current experiment contains the parameters you used to acquire the carbon-13 spectrum as outlined above.

The macro prints a help file in the text window of VNMR. You can ignore this if you wish, since we have already looked up the information in the on-line manuals.

f. **printon dg printoff**.

This prints a hard copy of the **dg** window after execution of the **dept** macro so that you can note the changes the macro made to the parameter set you used for your decoupled carbon spectrum.

Problem 45: Compare the listings of parameters before and after execution of the **dept** macro and explain all the differences you find.

g. dps

This displays a diagram of the pulse sequence showing the values of all the parameters directly affecting the pulse sequence. The display can be modified in various ways; if time permits, look up this command in the on-line manual to see how.

h. Note that pulses are being applied in the proton channel. Thus, at this point it is necessary to determine a value for **pp** and **pplvl** so that you can correctly set the proton pulse widths. This is done using the **ppcal** macro.

Join or create a new experiment and move your 1D carbon-13 parameters there, then type **ppcal**. This sets up a special pulse se-

quence for calibrating the proton pulse.

Note that you cannot use the proton pulse calibration determined while observing protons because the path of the radio frequency energy through the spectrometer is different when applying pulses through the decoupler channel than when they are applied through the observe channel and the strength of the $\mathbf{B}_2$ field may be different.

i. Read the help file that is printed by the macro and modify the parameters as needed. Check with your instructor as to the maximum **pplvl** that can be used with your spectrometer and probe. Set up the **pp** array from 0 to 40 microseconds to start with, then enter a more appropriate array, if necessary, when you have a better idea what the 90 degree pulse is. You may want to make an array with smaller steps near the 90 degree pulse value to get a more accurate determination. Save the data for your report.

j. pp=*x* mult=0.5,1.0,1.0,1.5 d1=2

Enter the value, *x*, determined in the previous step for the 90 degree proton pulse and collect a data set with the above multiplier array.

k. Save the data for later analysis.

l. In addition to the information you found in the on-line manuals, you can look at the source code for the pulse sequence you are using. The source may be found in **/vnmr/psglib** and may be viewed with your favorite editor. Even if you don't understand the actual code to generate the pulse sequence, the programmer often puts useful comments in the code. In the future, as you start using more complicated pulse sequences, the answers to your questions as to how the sequence works, what the parameters mean, what type of phase cycling is being used, etc. can be found in this source code.

Problem 46: From the source code listing for DEPT, what is the meaning of the parameter **mult**? Are your results consistent with the expected dependency of intensity as a function of the degree of protonation and the proton pulse width? Explain.

m. At a workstation learn to use the DEPT analysis macros and work up your data for your report. Also try experimenting with the interactive add subtract buffer to see if you can obtain similar results. (See the **add** command in the on-line manuals for a starting point.)

Problem 47: If you only have enough spectrometer time to take one DEPT spectrum, what value of **mult** would you use? Why?

Laboratory 6: Homonuclear Correlation Spectroscopy (COSY).

The COSY experiment yields much the same information as a homonuclear decoupling experiment in that scalar coupling networks are mapped out. However, a single COSY spectrum encompasses all the information which could be obtained from irradiating each multiplet in the spectrum and observing the changes in the other multiplets. In simple molecules this information could possibly be obtained more easily with a series of homonuclear decoupling experiments and in general where a few 1D experiments would give the needed information it is more efficient to obtain it that way. However, as molecules being investigated become larger and more complex, the COSY experiment can become the only reasonable way to get the needed data. Among other problems it is difficult to maintain adequate selectivity of the homonuclear decoupling so that in congested areas of the spectrum the irradiation will affect not only the multiplet of interest but also those nearby. The COSY experiment, on the other hand, will frequently unsort even overlapping multiplets.

In addition to the standard COSY pulse sequence used in this experiment, there also exist related pulse sequences such as double quantum COSY (DQCOSY) and phase sensitive COSY (COSYPS). To keep the acquisition time reasonable the 2D experiment run during this session will be preformed under fairly low resolution conditions in both dimensions, hence, the ability to resolve correlated peaks in heavily congested areas of the 2D spectrum will be greatly diminished and will not demonstrate the full potential of this type of experiment, but you should realize that even small proteins have been successfully analyzed by these methods. Although quantitative coupling information is present in the COSY spectrum, and coupling patterns can be observed with high enough digital resolution, it is generally better to use the phase-sensitive DQCOSY experiment for this purpose. (See Laboratory 7: on page 115.)

6.0.1. PROCEDURE:

a. You will need two experiment files for this procedure, one for your 1D “reference spectrum” and another for your 2D data.

b. Insert the sample provided by your instructor, set up the spectrometer for protons, acquire a spectrum, and narrow the sweep width to a practical minimum. (You will normally be provided a concentrated sample for carbon-13 experiments and a more dilute one for proton experiments. Be sure to use the dilute sample because the signal is too strong from the concentrated sample for this proton experiment.) Shim the magnet as necessary to obtain good line shape; highest

possible resolution is not required for this experiment. After the spectral width has been minimized acquire a spectrum using a 90 degree pulse width, **nt =** 16. Decrease the **gain** if error messages such as **ADC OVERFLOW** or **RECEIVER OVERFLOW** occur. This procedure will prevent similar problems from disrupting the 2D data acquisition.

c. Join the second experiment and move the parameter set from your setup spectrum to this file with the **mp** command.

d. cosy

This macro makes all the changes to the parameter set necessary to run the COSY experiment, but all the values may not be appropriate for your experiment. Note the new parameters in both **dg** and **dg1.** Critically examine the parameters and make any alterations you think are appropriate. Each increment must complete the full phase cycle as required by the pulse sequence to obtain satisfactory results. Find out what the minimum phase cycle is from the on-line manuals or the pulse sequence source code. (Note that the pulse sequence name is not cosy.) Enter 90 degree pulse widths for **PW** and **P1**, assume a T_1 of one second, make sure **d2** is zero, and set **sw1** equal to **sw** as determined in the set up proton spectrum. Set **fn** in both dimensions to 1 K and use 128 for the number of increments, **ni**. The delay, **d2**, is the t_1 delay. It is incremented by the program, and the increment is calculated from **sw1**. This is not an ordinary array such as you used when you measured T_1's and cannot be displayed; it is referred to as an “implicit” array.

e. pseudo

This macro enters pseudo echo convolution functions in both dimensions based on the acquisition times in t_1 and t_2. The values determined should be roughly correct but probably can be further optimized. You may want to experiment with other weighting functions later on the workstation.

f. time

The program will calculate and display the approximate total experiment time. If everything is set up correctly, this should not be more than ten or twenty minutes.

g. **go**

h. wft2d

While the acquisition is in progress, you may transform the partially completed 2D FID and observe how the resolution in the ν_1 dimension increases as more increments are collected.

i. foldt

This command "folds" the transformed spectrum about the diagonal and has the effect of helping to eliminate t_1 noise and spurious peaks in the spectrum. The **foldt** command requires that **sw** = **sw1**.

j. full

This command will set the chart parameters to display the contour plot so as to fill the screen.

k. dconi

Learn how to use this program to display vertical and horizontal traces and projections, and make expansions.

l. Save both the 1D and 2D data sets.

m. Arrange for a session on the workstation. Learn to use the interactive weighting program, **wti**, and get some additional practice with the features of the **dconi** program. Make a contour plot with the 1D reference spectrum plotted on the top and side with proper chemical shift scales. Be sure the chemical shifts in the 1D spectra align properly with those of the 2D spectrum. You may want to look up the **plcosy** macro. Discuss in your report how the information from the contour plot can be used to assign the spectrum.

Laboratory 7: Double Quantum Filtered Homonuclear Correlation Spectroscopy (DQCOSY).

The DQCOSY experiment is essentially similar to the COSY experiment in that scalar coupling networks are mapped out. However, it is particularly well adapted to being run as a hypercomplex, phase sensitive experiment.

Read the introductory material for the COSY experiment, Laboratory 6: on page 111, if you have not already done so.

A phase sensitive variant of the COSY experiment exists, but has the disadvantage that the peaks along the diagonal are in dispersion mode when the cross-peaks are in absorption mode. Thus, the intense tails of the diagonal can interfere with interpretation of the cross-peaks.The phase sensitive DQCOSY experiment can be phased so that all the peaks, including the diagonal, are in pure absorption, either positive or negative. With a phase sensitive display and reasonable digital resolution, it becomes evident that the cross-peaks have antiphase character with respect to the direct couplings, but not for the indirect or passive couplings. (See Derome beginning on page 217 for an excellent discussion of how to interpret these patterns.) Beware of antiphase peaks that partially or completely cancel each other. Full analysis of the coupling constants will not be required for this experiment, but the resolution in your spectrum will be sufficient to permit this if you wish to do it on your own.

7.0.1. PROCEDURE:

a. You will need two experiment files for this procedure, one for your 1D “reference spectrum” and another for your 2D data.

b. Insert the sample provided by your instructor, set up the spectrometer for protons, acquire a spectrum, and narrow the sweep width to a practical minimum. (You will normally be provided a concentrated sample for carbon-13 experiments and a more dilute one for proton experiments. Be sure to use the dilute sample because the signal is too strong from the concentrated sample for this proton experiment.) Shim the magnet as necessary to obtain good line shape. After the spectral width has been minimized acquire a spectrum using a 90 degree pulse width and **nt =** 16. Decrease the **gain** if error messages such as **ADC OVERFLOW** or **RECEIVER OVERFLOW** occur. This procedure will prevent similar problems from disrupting the 2D data acquisition.

c. Join the second experiment and move the parameter set from your setup spectrum to this file with the **mp** command.

d. dqcosy

This macro makes all the changes to the parameter set necessary to run the DQCOSY experiment, but all the values may not be appropriate for your experiment. Note the new parameters in both **dg** and **dg1.** Critically examine the parameters and make any alterations you think are appropriate. Each increment must complete the full phase cycle as required by the pulse sequence to obtain satisfactory results. Find out what the minimum phase cycle is from the on-line manuals or the pulse sequence source code. Be sure **pw** is set to the correct 90 degree pulse width. Since signal-to-noise will normally not be a problem on this sample, it is acceptable to saturate the spins substantially; use a **d1** of 0.6 sec. Note that **hs** (the homospoil parameter) is set to 'yn'. Because we will be pulsing very rapidly, this is needed to destroy any excess transverse magnetization before the next FID is generated. This will disturb the lock, so you must turn up the lock power and gain high enough so that lock is not lost. The lock will still work sufficiently well that we will get good data. Make sure **d2** is zero, and set **sw1** equal to **sw** as determined in the set up proton spectrum. Set **np** to 1024 and **ni** to 512. Set **fn** in both dimensions to 2 K. The delay, **d2**, is the t_1 delay. It is incremented by the program, and the increment is calculated from **sw1**. This is not an ordinary array such as you used when you measured T_1's and cannot be displayed; it is referred to as an "implicit" array.

e. time

The program will calculate and display the approximate total experiment time. If everything is set up correctly, this should be about two hours.

f. **go**

g. wft2da

While the acquisition is in progress, you may transform the partially completed 2D FID and observe how the resolution in the ν_1 dimension increases as more increments are collected. Use a gaussian convolution function of appropriate width to alleviate truncation wiggles in both dimensions. Use **wti** to determine the correct weighting function. Use **wft1da** prior to **wti** to set the convolution function in the ν_1 dimension.

h. foldt

This command "folds" the transformed spectrum about the diagonal and has the effect of helping to eliminate t_1 noise and spurious peaks in the spectrum that don't possess symmetry about the diagonal. The **foldt** command requires that **sw** = **sw1**.

i. dconi

Learn how to use this program to display vertical and horizontal traces and projections, and make expansions.

j. Save both the 1D and 2D data sets.

k. Arrange for a session on the workstation. Learn to map out the scalar coupling networks, and get some additional practice with the features of the **wti** and **dconi** programs. Make a contour plot with the 1D reference spectrum plotted on the top and side with proper chemical shift scales. Be sure the chemical shifts in the 1D spectra align properly with those of the 2D spectrum. You may want to look up the **plcosy** macro. Discuss in your report how the information from the contour plot can be used to assign the spectrum.

Laboratory 8: Heteronuclear Chemical Shift Correlation Spectroscopy (HETCOR).

The determination of the structure of molecules often requires the use of several different kinds of information. Like a jigsaw puzzle, each puzzle piece alone does not give a clear picture; but, when all the pieces are assembled properly, the picture becomes clear. If one begins with a 1D proton and a 1D carbon spectrum of the molecule to be studied, some things can be inferred about the structure, but the picture is by no means complete. The DEPT data from Laboratory 5 tells us how many protons are attached to each carbon. One can add the proton-proton correlation from Laboratory 6 or 7 to show which protons are near each other through bonds, but still not everything is known about the structure. In this laboratory session we will determine which proton is attached to which carbon. Thus, whatever we have learned about the protons can be correlated with the carbons. The reverse is also true; the greater chemical shift dispersion for carbon (and for many other X nuclei as well) in conjunction with spreading of the peaks into the second dimension permits the assignment of proton resonances in complicated congested spectra in cases where assignment of the 1D proton spectrum is impossible. The upfield region of a proton spectrum often very crowded, but the corresponding region of a carbon-13 spectrum is usually well resolved.

In this experiment the one bond scalar interaction is used to set up the chemical shift correlations, however, by appropriate adjustment of the mixing delays, long range interactions can be emphasized. This can be useful in the assignment of quaternary carbons. In addition to the HETCOR sequence several modifications exist that are used less routinely than the basic HETCOR sequence. Two examples that are well suited to investigate long range chemical shift correlations via long range scalar couplings include XCORFE, COLOC, and the so called relayed coherence transfer experiments.

8.0.1. PROCEDURE:

a. For this laboratory we suggest that you use three experiments, one for your reference proton spectrum, one for the reference carbon spectrum, and a third for the 2D data. Collect the carbon spectrum with full proton decoupling and for both carbon and proton use the smallest **sw** that is practical without peaks aliasing from outside the spectral window; or, if peaks must be aliased (e.g., solvent peaks) adjust **sw** and **tof** so that the aliased peaks will appear in a region free of signals of interest. (You will normally be provided a concentrated sample for carbon-13 experiments and a more dilute one for proton experiments. Be sure to use the concentrated sample for this experiment.)

b. Acquire the 1D carbon spectrum. You should be able to use shims from one of your previous experiments. However, you can shim on the dioxane sample if necessary.

c. Join a second experiment and retrieve the 1D proton spectrum that you acquired in Laboratory 6 or 7. If you don't have this, you will need to acquire a reference proton spectrum.

d. Join the third experiment and move the carbon acquisition parameters to it.

e. hetcor

The setup macro for the HETCOR pulse sequence loads the new acquisition parameters as well as the proper pulse sequence. The macro will query you for the location of the proton reference spectrum, or you can supply it as an argument to the macro. Use **nt** = 8 and appropriate values for **pp** and **pplvl** from Laboratory 5. Set **pw** and **tpwr** for a 90 degree carbon pulse. If you don't have a reliable calibration, check it on the sample provided for this experiment. Assume a proton T_1 of two seconds, an average one bond coupling, **j1xh** of 140 Hz, and set the long range coupling parameter, **jnxh** = 0; you will do only one bond correlations in this exercise. Collect the data with decoupled H-H multiplets by setting **hmult** appropriately and use no presaturation pulses. Set **np** in the F_2 (carbon) dimension to 4096 and use 128 increments for **ni**. Set **fn** to 8192 and **fn1** to 512.

f. go

You can experiment with weighting and transforming the data as the acquisition continues.

g. Be sure to save all three FID's.

At this point you can continue on a workstation, if necessary.

h. Use the appropriate commands to weight transform and plot your data. These data are not hypercomplex, so you must use the appropriate commands to analyze the data. Make a 2D contour plot with the corresponding 1D spectra along the appropriate axes. The macro **plhxcor** is useful for this. The command **dpcon** creates a screen display that is a true contour plot like the one produced by the **pcon** command rather than a color intensity map as is produced by the **dcon** or **dconi** commands. The **dpcon** command gives better definition of the contours as they will appear on a printer, but it can take a rather long time to compute this display, so use it with caution. Experiment first with a highly expanded portion of the spectrum, so that the computation time will not be excessive. The various display and plotting commands have lots of options to control how the spectra are displayed or plotted; these can be found in the on-line manuals.

Learning to properly work up, and plot high quality 2D data takes practice. Read the manual and practice as much as you can.

Using the results from your **COSY** or DQCOSY data make as many assignments as possible in your **HETCOR** spectrum.

Laboratory 9: Indirect Detection of Heteronuclear Chemical Shift Correlation (HMQC, HMBC).

In contrast to the HETCOR experiment which uses the X nucleus (^{13}C in this case) as the observed nucleus, the HMQC (Heteronuclear Multiple Quantum Correlation) experiment acquires proton FID's detecting the carbon frequencies indirectly. This method has the advantage that protons can be detected with substantially greater sensitivity per nucleus than is possible for carbon or other X nuclei of lower gyromagnetic ratio. There are several drawbacks to this method: (1) It is necessary, usually, to cover a rather wide spectral region in the indirectly detected dimension which requires many t_1 increments to achieve high resolution. However, it should be noted that, often, modest resolution is sufficient. (2) If the X nucleus is of low abundance (1.1% natural abundance for ^{13}C , for example) the proton signals from molecules with no ^{13}C must be suppressed. (3) If decoupling is required, it is more difficult to decouple over the broad chemical shift range of ^{13}C than over the comparatively narrow one for protons. In spite of these obstacles, the HMQC experiment has become widely used, and modern spectrometers are capable of doing it well.

The determination of the structure of molecules often requires the use of several different kinds of information. Like a jigsaw puzzle, each puzzle piece alone does not give a clear picture; but, when all the pieces are assembled properly, the picture becomes clear. If one begins with a 1D proton and a 1D carbon spectrum of the molecule to be studied, some things can be inferred about the structure, but the picture is normally not complete. The DEPT data tells us how many protons are attached to each carbon. One can add the proton-proton correlation from COSY or DQCOSY spectra to show which protons are near each other through bonds, but still not everything is known about the structure. In this laboratory session we will determine which proton is attached to which carbon. Thus, whatever we have learned about the protons can be correlated with the carbons. The reverse is also true; the greater chemical shift dispersion for carbon (and for many other X nuclei as well) in conjunction with spreading of the peaks into the second dimension permits the assignment of proton resonances in complicated congested spectra in cases where assignment of the 1D proton spectrum is impossible. The upfield region of the proton spectrum is often very crowded, but the corresponding region of the carbon-13 spectrum is usually well resolved.

In the first section of this experiment the one bond scalar interaction is used to reveal which proton is bonded to which carbon. In the second section the multiple bond scalar interactions are used to reveal which carbons are nearby to a given proton. This latter experiment is often referred to as HMBC (Heteronuclear Multiple Bond Correlation).

9.0.1. PROCEDURE:

a. If necessary, change the probe to an appropriate indirect detection probe. Load appropriate shims and verify line shape as needed.

b. For these 2D experiments it is necessary to know the calibration of carbon pulses applied through the decoupler channel. This calibration is difficult to accomplish using natural abundance compounds, so a standard sample containing carbon-13 enriched methyl iodide is provided. Obtain 1D coupled carbon spectrum of the provided standard sample. this can be done through the decoupler coil of your indirect detection probe. Note that the chemical shift of methyl iodide is upfield from TMS. Determine the value of **tof** that will place the methyl iodide quartet exactly at the center of your spectrum. Record this value of **tof** for later use. Ignore the other peaks in the spectrum. These compounds are used for other calibrations.

c. Obtain a proton spectrum of the standard sample and place the methyl iodide peaks near the center of the spectrum. The methyl iodide signals are the three equally spaced peaks of about equal intensity. Ignore the other peaks. The center peak is from carbon-12 methyl iodide, and the outer peaks are the doublet from the carbon-13 methyl iodide. Calibrate the proton 90 degree pulse.

d. pwxcal

This macro sets up a simple magnetization transfer experiment. Set all the parameters appropriately, then array the carbon pulse from zero to 50 microseconds. Ask your instructor what power is acceptable for the probe you are using. Phase the first spectrum to give positive peaks. The center peak will remain constant throughout the array. The two outer peaks will decrease and pass through a null when the carbon pulse is 90 degrees. If necessary, reset the array and reacquire the data to accurately determine the carbon 90 degree pulse.

e. Now you will need four experiments, one for your reference proton spectrum, one for the reference carbon spectrum, a third for the HMQC data and a fourth for the HMBC data. For both carbon and proton use the smallest **sw** that is practical without peaks aliasing from outside the spectral window; or, if peaks must be aliased (e.g., solvent peaks) adjust **sw** and **tof** so that the aliased peaks will appear in a region free of signals of interest. (You will normally be provided a concentrated sample for carbon-13 experiments and a more dilute one for proton experiments. Be sure to use the dilute sample because the signal is too strong from the concentrated sample for this proton experiment.)

f. Acquire the 1D proton spectrum.

g. Join a second experiment and retrieve the 1D carbon spectrum that you acquired in Laboratory 5. If you don't have this, you will need to acquire a reference carbon spectrum using the more concentrated sample. This spectrum could be acquired using the dilute sample, but it would probably take overnight. Be aware that there may be a small concentration dependent change in chemical shifts when spectra taken at different concentrations are used.

h. Join the third experiment and move the proton acquisition parameters to it.

i. hmqc

This setup macro for the HMQC pulse sequence loads the new acquisition parameters as well as the proper pulse sequence. By default the macro will set up for carbon as the X nucleus. For other X nuclei you can supply an argument to the macro. Set **sw1** and **dof** to the values of **sw** and **tof,** respectively, from your carbon reference spectrum. For this experiment we will not use ^{13}C decoupling, so set **dm** to 'n' and **dmm** to 'c'. Use **nt =** 8 and the values for **pwx** and **pwxlvl** that you just determined. Set **pw** and **tpwr** for a 90 degree proton pulse. If you don't have a reliable calibration, check it on the sample provided for this experiment. Assume a proton T_1 of two to three seconds unless you know a better value from previous work on this sample, an average one bond coupling, **j**, of 140 Hz, and set the long range coupling parameter, **jnxh** = 0; we want one bond correlations for this spectrum. Use no presaturation pulses. Set **np** in the F_2 (proton) dimension to 4096 and use 512 increments for **ni**. Set **fn** = 4096 and **fn1** = 2048. Adjust d1 to an appropriate value for the relaxation time of the protons in your sample. Check that the total acquisition time is reasonable for the time you have available.

j. The value of **null** may be optimized for slightly better suppression of the signals from carbon-12 molecules by moving these parameters to some other experiment, setting **nt** = 1, **ni** = 1, **phase** = 1, and making an array of **null** over 0.1 to several seconds. the spectra obtained will be dominated by the proton signals from the carbon-12 molecules. Choose the value of **null** that gives minimum average amplitude of the spectrum. Return to the HMQC experiment and set the best value of **null**.

k. go

You can experiment with weighting and transforming the data as the acquisition continues, but go on immediately to the next step. Do not wait for the HMQC experiment to complete.

l. Join another experiment and move the parameters from the HMQC experiment to the new one.

m. Make the following changes to the parameters: **mbond** = 'y',

null = 0, **taumb** = 0.055. Be sure that the total acquisition time is reasonable for the time you have available.

n. go

Since the HMQC experiment has probably not yet completed, this HMBC experiment will be queued and will start acquisition as soon as the experiment in progress complete acquisition.

o. Be sure to save all four FID's.

At this point you can continue on a workstation, if necessary.

p. Use the appropriate commands to weight transform and plot your data. These data are hypercomplex, so you must use the appropriate commands to analyze the data. Make a 2D contour plot with the corresponding 1D spectra along the appropriate axes. The macro **plhxcor** is useful for this. The command **dpcon** creates a screen display that is a true contour plot like the one produced by the **pcon** command rather than a color intensity map as is produced by the **dcon** or **dconi** commands. The **dpcon** command gives better definition of the contours as they will appear on a printer, but it can take a rather long time to compute this display, so use it with caution. Experiment first with a highly expanded portion of the spectrum, so that the computation time will not be excessive. The various display and plotting commands have lots of options to control how the spectra are displayed or plotted; these can be found in the on-line manuals.

Learning to properly work up, and plot high quality 2D data takes practice. Read the manual and practice as much as you can.

Using the results from your COSY or DQCOSY data make as many assignments as possible in your HMQC and HMBC spectra.

Laboratory 10: Heteronuclear Two Dimensional J–Spectroscopy.

In previous 2D experiments such as COSY and HETCOR correlations were made between two chemical shifts connected by some interaction, such as, scalar coupling; in J–spectroscopy the emphasis is on interpretation of multiplet structure. Pulse sequences for heteronuclear and homonuclear J–spectroscopy, however, both refocus chemical shifts but display scalar coupling. For the former chemical shift and scalar coupling can be made completely orthogonal by decoupling protons during t_2. Homonuclear J–spectroscopy, on the other hand, does not completely separate the two because broadband decoupling cannot be preformed during t_2, but can be made to appear so after appropriate data manipulations. Magnet inhomogeneity effects are also refocussed, so that natural line widths free of inhomogeneity broadening can be obtained if the data are acquired with sufficient digital resolution. This permits resolution of couplings that would be impossible in a 1D spectrum. Severe overlap of multiplets resulting from scalar coupling can make the interpretation of 1D experiments difficult if not impossible.

10.0.1. PROCEDURE:

a. (You will normally be provided a concentrated sample for carbon-13 experiments and a more dilute one for proton experiments. Be sure to use the concentrated sample for this experiment.) For this session set up three experiments, two for 1D spectra and a third for the 2D data. In one experiment run a minimum spectral width ^{13}C spectrum of the more concentrated sample with full proton decoupling and 32 K data points. In another experiment use the same acquisition parameters modified so as to collect a proton coupled ^{13}C spectrum with NOE as was performed in Laboratory 3: on page 95. This second experiment is for demonstration purposes only to compare the results of the 1D and 2D methods.

b. Move the carbon observe parameters to the experiment where you want to do the 2D; then use the **het2dj** setup macro. Review the documentation for hints on how to set the experiment up. Set up the acquisition with the following considerations: run the gated decoupler mode, **sw1** = 500 Hz, use WALTZ decoupling, and **nt** a multiple of 4. Be sure the number of transients used is sufficient to give reasonable signal to noise in a 1D spectrum. Repetition rates are governed by the carbon T_1's that you determined in Laboratory 2: on page 85. Set the number of increments **ni** so as to have a total run time of about one hour. The pulse sequence library (**psglib**) and the on-line manuals, as you found in the past, contains many of the answers to your questions concerning new parameters for the pulse sequences

you are using; get used to using it.

c. **go**

Remember to save your data.

d. **wti, wft2d,** etc.

Interactively weight and transform your data.This is not hypercomplex data.

e. foldj

The **FOLDJ** command will make the data symmetric by folding about the center of the F1-axis of your data.

f. plhet2dj

Plot the 2D data with the 1D carbon spectrum along the F_2-axis. Obtain traces of any two multiplets found along F_1 and plot separately. Clearly label the spectrum as to the source of the plot, i.e., the trace number, the carbon shift it represents, etc.

g. proj(...), trace, ds, etc**.**

Experiment with projections and traces. Include some of them in your report.

Laboratory 11: ^{13}C Two Dimensional INADEQUATE, Direct Detection of Carbon-Carbon Bonds.

In this experiment you will obtain a 2D INADEQUATE spectrum of the molecule you have been studying. The experiment is discussed in detail in the lecture notes, section 15.2. on page 63. This experiment will employ the hypercomplex or States, Haberkorn, and Ruben method for producing phase sensitive 2D data that does not have the phase twist line shape problem. You may also want to review section 14.3. on page 55 of the lecture notes. The data from this experiment will allow you to assemble the complete carbon skeleton of the molecule. You will need more than the usual amount of time to acquire the data for this experiment. Consult your instructor before making your instrument reservation.

11.0.1. PROCEDURE:

a. Set up the spectrometer in the way that you should know well by now and obtain a decoupled ^{13}C spectrum. (You will normally be provided a concentrated sample for carbon-13 experiments and a more dilute one for proton experiments. Be sure to use the concentrated sample for this experiment.) Limit the spectral width to contain only the lines of interest and the solvent lines. Make sure that the magnet is well shimmed and that the decoupler is set up correctly and well calibrated. The S/N in the 2D spectrum will be rather poor and any degradation of the lines due to poor shimming or poor decoupling may cause your data to be partially uninterpretable. You should be able to obtain a line width of about 0.3 Hz with good line shape for most small molecules. Check the 90 degree pulse calibration also.

b. Move the parameters to another experiment and execute the **inadqt** macro. This macro as supplied by Varian sets many parameters to nonoptimal values for our purposes. Make the following changes:

   np = 32768 This should give an **at** of about 3 s if **sw** is set correctly.

   sw1 = **sw**

   Be sure **pw** is set to a carefully calibrated 90 degree pulse.

   d1 = ‘n’

   nt = 16

   ni = 64

   dp = ‘y’

c. If everything is set up correctly you should be able to use the **time** macro to verify that the acquisition will require less than 3 hr.

d. Start the acquisition with **go**.

e. While the acquisition is progressing you can Fourier transform the partially completed data using **wft1da** and **wft2da**. Be sure to set **fn** and **fn1** appropriately. Use **wti** to set up the convolution functions. Gaussians work quite well for this data set. You may want to set **pmode** = 'full'. Look up the **pmode** parameter and learn what it does.

f. When the 2D FID is about half complete you should begin to be able to see bond patterns. Since signals can occur only near the chemical shifts that you know from the 1D spectrum, it works well to examine about a two ppm wide strip in the chemical shift direction in the vicinity of each 1D shift while maintaining full spectral width in the double quantum direction. Look for the characteristic antiphase doublets. When you find one look for its mate at the same double quantum frequency. Continue this process for all the lines in the 1D spectrum.

g. Save your data for later analysis.

Laboratory 12: Through Space Correlations (NOESY).

Read the introductory material for the COSY and DQCOSY experiments, Laboratory 6: on page 111 and Laboratory 7: on page 115, if you have not already done so.

The NOESY experiment is essentially similar to the COSY and DQCOSY experiments in its form and data processing protocols. The NOESY two-dimensional FID is hypercomplex and is processed essentially the same way as was the DQCOSY data in Laboratory 7: on page 115. It differs, however, in that dipole-dipole relaxation is the interaction to be exploited rather than scalar coupling. Since the dipole-dipole mechanism acts through space not through bonds, correlations indicate that the correlated nuclei are close through space, whether or not they are close through intervening bonds. The acronym, NOESY, stands for Nuclear Overhauser Effect Spectroscopy. Refer to Section 11. on page 41 for a discussion of the NOE and its relationship to the dipole-dipole relaxation mechanism.

How can the NOESY correlations be used? In a protein, for example, two protons that reside in two different amino acids widely separated in the polypeptide chain may show a NOESY correlation indicating that the chain is folded such that they are close to each other through space. In a given protein there may be dozens or even hundreds of NOESY correlations. Such correlations, taken together with other known structural constraints, can provide enough data to fully determine the three-dimensional structure of the protein. This information is very valuable because proteins generally do not perform their biological function unless they are properly folded, and knowing the way they are folded is essential to understanding how they function.

In this experiment we will be dealing not with proteins but with smaller molecules. In this case the NOESY correlations can be used to determine the geometry of stereo-centers, distinguish between cis and trans isomers, distinguish between axial-axial and axial-equatorial relationships in cyclohexyl rings, etc. The intensities of the cross-peaks in a NOESY spectrum of a small molecule are generally only one or two percent as large as the corresponding diagonal peaks and of opposite phase. Thus, if the diagonal peaks are phased for pure negative absorption, the cross-peaks will be phased for positive absorption. If a COSY response is also possible between two protons because they are close through bonds one will often see the COSY cross-peak superimposed on the NOESY cross-peak. One must be careful not to misinterpret a COSY response as a NOESY correlation. For this reason, it is useful to run a COSY for comparison with the NOESY spectrum.

12.0.1. PROCEDURE:

a. You will need two experiment files for this procedure, one for your 1D "reference spectrum" and another for your 2D data.

b. Insert the sample provided by your instructor, set up the spectrometer for protons, acquire a spectrum, and narrow the sweep width to a practical minimum. (You will normally be provided a concentrated sample for carbon-13 experiments and a more dilute one for proton experiments. Be sure to use the dilute sample because the signal is too strong from the concentrated sample for this proton experiment.) Shim the magnet as necessary to obtain good line shape. After the spectral width has been minimized acquire a spectrum using a 90 degree pulse width and **nt =** 16. Decrease the **gain** if error messages such as **ADC OVERFLOW** or **RECEIVER OVERFLOW** occur. This procedure will prevent similar problems from disrupting the 2D data acquisition.

c. Join the second experiment and move the parameter set from your setup spectrum to this file with the **mp** command.

d. noesy

This macro makes all the changes to the parameter set necessary to run the NOESY experiment, but all the values may not be appropriate for your experiment. Note the new parameters in both **dg** and **dg1.** Critically examine the parameters and make any alterations you think are appropriate. It may be useful to display the pulse sequence using **dps**.

The **mix** parameter is the most critical one for this experiment. This is the time during which the NOE is allowed to build up before it is measured. The cross-peak intensities increase with increased **mix** values up to about T_1 of the protons then decreases exponentially to zero as **mix** is increased further. If time permits run a quick T_1 measurement on your sample, and use about 0.1 to 0.5 times T_1 for **mix**. Otherwise ask your instructor for an appropriate value. There will probably not be time to do so during this session, but an array of values of mix may be run to determine the rate at which the NOE grows. The closer two protons are the faster the NOE will build up, so that internuclear distances can be, at least roughly, estimated from the initial slope of the buildup curve. However, with only one value of mix, one can still conclude from the presence of a NOESY cross-peak that two protons are within a few Angstroms of each other. For example, cis protons generally will give a NOESY cross-peak, trans protons will not.

Each increment must complete the full phase cycle as required by the pulse sequence to obtain satisfactory results. Find out what the minimum phase cycle is from the on-line manuals or the pulse se-

quence source code. Be sure **pw** is set to the correct 90 degree pulse width. Use a **d1** substantially longer than the **mix** time. Three or more T_1's is desirable if time permits. Make sure **d2** is zero, and set **sw1** equal to **sw** as determined in the set up proton spectrum. Set **np** to 2048 and **ni** to 256. This will give adequate resolution except for the most demanding cases. Set **fn** in both dimensions to 2 K. The delay, **d2**, is the t_1 delay. It is incremented by the program, and the increment is calculated from **sw1**. This is not an ordinary array such as you used when you measured T_1's and cannot be displayed; it is referred to as an "implicit" array.

e. time

The program will calculate and display the approximate total experiment time. Make sure the experiment will complete in the time you have been allowed. If not, you may have to reduce **ni** or **d2**.

f. **go**

g. wft2da

While the acquisition is in progress, you may transform the partially completed 2D FID and observe how the resolution in the ν_1 dimension increases as more increments are collected. Use a gaussian convolution function of appropriate width to alleviate truncation wiggles in both dimensions. Use **wti** to determine the correct weighting function. Use **wft1da** prior to **wti** to set the convolution function in the ν_1 dimension.

h. dconi

Learn how to use this program to display vertical and horizontal traces and projections, and make expansions.

i. Save both the 1D and 2D data sets.

j. Arrange for a session on the workstation. Find all the NOESY cross-peaks and see what they tell you about the stereo configuration of your molecule. If desired, you can get some additional practice with the features of the **wti** and **dconi** programs. Make a contour plot with the 1D reference spectrum plotted on the top and side with proper chemical shift scales. Be sure the chemical shifts in the 1D spectra align properly with those of the 2D spectrum. You may want to look up the **plcosy** macro; it can be used just as you did in the COSY or DQCOSY experiments

Part III
Appendices

These appendices are designed to supplement the spectrometer User Manual. More detail can be found there on many of the items discussed in these appendices. Spectrometer commands and parameters are given in bold type for easy identification.

Appendix 1: Locking and Shimming the Spectrometer.

1.1. Obtaining Lock.

If a high field NMR spectrometer is to produce satisfactory results, the ratio of the field strength, B_0, to the frequency, ω_0, must remain constant to within about one part in 10^{10}, often for hours or even days at a time for long time averaging. This extraordinary stability can be achieved by doing an NMR experiment and using the result in a feed back system. This is called an NMR lock.

Additional details concerning locking and shimming may be found in the vnmr manual “Getting Started” Chapter 6.

It is common practice to make up samples for proton NMR spectroscopy in solvents with most (usually greater than 99.5%) of the protium replaced by deuterium so that the strong proton signals from the solvent will not interfere with those from the solute of interest. Having done this, one can use the deuterium NMR signal to produce the required field-frequency lock. This type of lock is called an internal lock and is the most common type used for high resolution liquids NMR. Deuterated solvents are almost universally used for NMR samples even when a nucleus other than ^{1}H is being observed. The fluorine-19 resonance is also used but less frequently. It has been found to be of great value when very low temperature liquid NMR studies are being carried out using low freezing point freon type solvents or when the spectrometer is set up to observe deuterium. It is generally not practical to observe and lock on deuterium simultaneously. The lock nucleus can also be contained in an ampule external to the sample of interest. This is termed an external lock and is much less commonly used for high resolution liquids work.

While searching for an NMR lock signal (using the **acqi** window) you are actually doing an NMR experiment varying the field with the **z0** knob while periodically pulsing the sample using the lock transmitter. The trace on the screen is the deuterium FID following each pulse. The field is adjusted until the resonance condition is achieved. At a field of 7.05 Tesla, for example, this occurs at 46.05 MHz. The lock signal, when off resonance, appears as a sinusoidal trace. The frequency of this sinusoid is the difference between the lock transmitter frequency and the Larmor frequency of the deuterium. One must adjust **z0** until this difference is near zero. Thus, as resonance is approached, fewer cycles of the FID are seen but with greater amplitude. Signals far from resonance will be of low amplitude with many cycles visible. When **z0** is adjusted to the on-resonance condition, the feedback system can be activated by pressing the “lock on” button. The signal then ceases to oscillate

at all and any drift in frequency or field will be compensated to maintain a constant ratio. Adjust the lock phase for maximum lock level.

When searching for a lock signal high values for both lock power and gain may be used. Locking on a signal under these conditions may result in an unstable oscillating lock signal. This is sometimes referred to as a “breathing” lock. It is caused by saturation of the lock nuclei as a consequence of too much lock power. When this occurs, slowly reduce the lock power and increase the lock gain, if necessary, until the oscillations stop. As a rule it is better to use lower lock power and higher lock gain to achieve an adequate lock signal than the reverse. If too much lock gain is used, however, the signal becomes noisy and the lock unstable. The values used will depend upon the concentration and T_1 of the deuterium in the sample. After a little experience with your samples, you will know the minimum lock solvent concentration necessary to produce an adequate lock. For shimming it is best to have very little saturation of the lock; for acquisition some saturation can be tolerated as long as the lock is stable. Lock saturation can be checked by noting the lock level then decreasing the lockpower by six units. With no saturation the lock level should decrease to half its former value. If it is greater than half by more than about three units of lock level, increase the lock gain a few units and repeat the process by decreasing the lockpower by six more units. If the shims are far from optimum, you may need to accept less than optimum power and gain settings until after you have shimmed the magnet a little better. If you cannot achieve a stable unsaturated lock after shimming, then you may want to increase the deuterium content of your sample. If this is impossible, you may want to just proceed anyway with the best compromise you can achieve. But, be aware that you are operating under nonoptimal lock conditions, and you may encounter difficulty shimming, with consequent decrease in the quality of your spectra.

If the system will not lock using sample with a strong lock signal, it is usually because the lock phase is too far from the correct value. Change the phase by about 90 degrees and try again. With a weak lock it is sometimes necessary to lock on a standard sample with a strong lock, set the lock phase correctly, then return to the original sample. Once locked the signal can be accurately phased by maximizing the lock amplitude as a function of the lock phase. It is also possible to have the computer perform these operations automatically using the **lock** command, but you should learn to do it manually because manual locking is usually faster and more reliable. The computer algorithm does not always work for weak lock signals.

If you have trouble locking and shimming on your sample, the best strategy is to put in either a proton or a carbon-13 lineshape sample, and see if you can achieve lineshape about as good as that of the stored standard spectra. If you can, the problem is most likely with your sample or tube. If you cannot, you should seek help from one of the NMR staff.

1.2. Shimming the Magnet.

A superconducting magnet differs from permanent and electromagnets in that the direction of the field is parallel to the long axis or spinning axis of the sample tube rather than perpendicular to it. Thus, the spinning gradients are the ***z*-**axis shims and the nonspinning gradients are the ones involving *x* or *y*. Because the shim coils cannot be made perfectly, a change in one gradient will affect the optimum value of others. For example, the **z4** gradient contains a considerable component of **z2** so that, whenever **z4** is adjusted, **z2** must be readjusted to compensate. Hence, it is necessary to cycle through sets of gradients until all are simultaneously maximized. Additionally, the lock phase may need to be reoptimized as the lineshape improves. You will find that there are as many methods used to shim a magnet as there are operators. Shimming magnets, requires some experience as well as training.

It is difficult to write down exactly how to shim a magnet just as it is to explain how to hit a golf ball straight down the fairway. Certain basic principles can be explained; then you just have to do it until you can do it well. A page indicating how misadjustment of various shims affects the lineshape is posted near each spectrometer. Be careful not to take these diagrams too literally because different magnets behave differently, but the general principles are correct. Remember also that these diagrams represent gross missetting of a single gradient. If several gradients are misset, the result can be more difficult to interpret. Trust standard shim settings. Under normal circumstances with a sample containing no paramagnetic materials, that completely fills the coil (50 mm liquid height in the tube) in a high quality tube, only **z1**, and **z2** will change much from sample to sample or even from week to week. If you seem to be experiencing dramatic changes in the other shims, chances are something else is wrong that can't be fixed by merely adjusting the shims. If you don't immediately get good results on your unknown sample, go to a standard lineshape sample. If you can't get an adequate spectrum of a lineshape standard you can't hope to get a good spectrum of an unknown. Conversely, if you do get a good standard spectrum, you will usually get a good spectrum of an unknown.

Appendix 2: Sample Preparation

The importance of proper sample preparation can not be over emphasized. The standard samples used in these exercises for spectrometer performance checks were made with high purity solvents, and solutes, and high quality sample tubes. Paramagnetic impurities, including dissolved oxygen, should be rigorously excluded from a sample for best results. The levels of dissolved oxygen encountered by preparing samples in ambient atmosphere are not of much concern when chemical shifts and coupling constants are the object of the experiment; but, if any sort of relaxation or NOE data is desired, oxygen must be excluded. Paramagnetic ions can have a dramatic effect on your spectrum, even at very low concentrations. This is especially true if the ions tend to complex with the compound of interest. Non-homogeneous or viscous samples can seriously degrade resolution.

High quality sample tubes such as Wilmad 528-PP or equivalent tubes are of acceptable quality. Wilmad 535-PP or 545-PP tubes may be used for the best proton work. Sample tubes should never be cleaned with cleaning solutions such as potassium dichromate because the glass will be impregnated with paramagnetic chromium. Also, do not dry your tubes in a hot drying oven because the thin walls of an NMR tube can warp. Some cheaper sample tubes often have enough camber to be beyond the close tolerances allowed between the sample tube and the receiver coil in the probe. This will result in difficulty in spinning, will score the tube, and may damage the probe. Take care not to scratch your tubes while cleaning them. A scratched tube is likely to break in the probe. Test your tubes by dropping them from a height of about 10 cm bottom first on a solid countertop; a sound tube will not break.

If a sample does not spin properly once inserted in the probe eject the sample, clean the spinner turbine and sample tube, then try again. Never touch the lower section of the turbine which is in contact with the air bearings of the spinner housing. Handle only the upper larger diameter part of the turbine, or, better yet, handle it with a kimwipe. Oils from your fingers accumulate on the bearing surfaces inside the probe eventually causing the samples not to spin. With these precautions, if the sample still does not spin, try a standard sample. If the standard does not spin, consult one of the NMR staff. If the standard spins, the problem is with your tube and a different one should be used. Never use any kind of sample container not explicitly designed for NMR use as a sample tube without approval from the laboratory manager. Spin rates should be on the order of 15-25 Hz for 5 mm sample tubes.With samples properly prepared as described below, vortexing of the sample is

not a problem. With 10 mm sample tubes spin rates greater than 15 Hz will form a vortex that penetrates into the coil region of the probe and destroys magnetic field homogeneity.

Sample volumes should be kept constant from sample to sample and should be 50 mm in height from the bottom of the tube to the meniscus. A volume of 0.7 mL will produce the correct height in standard 5 mm NMR tubes. Accurately adjusting the volume of your research sample to equal that of the standard sample used to obtain the standard shims will make it significantly easier to shim on your research sample beginning from the standard shim settings. The increase in solute concentration by making a “short” sample usually turns out not to be a significant advantage because the best lineshape is difficult to achieve. The ideal sample would be a perfect sphere; but, since this is practically unachievable, a cylinder that extends from well below the receiver coil to well above it is a reasonable alternative. Short samples produce artifacts in the lineshape that are difficult or impossible to shim out.

Special cells and probes are available to deal with very limited sample amounts. You may want to discuss your problem with one of the NMR staff when you encounter this situation.

Samples should be inserted into the spinner turbine using the depth gauge provided. Accurate positioning of the sample in the coil is as important as correct sample height for maintaining good homogeneity without excess labor for shim adjustment. Standard sets of shims and shim settings recalled from previously collected data will be of much greater value when samples are prepared in a consistent fashion.

Appendix 3: Solutions to Problems.

The following are solutions to the problems in the lecture notes.

Problem 2: on page 6

$$\mathbf{M} = \sum_i \boldsymbol{\mu}_i$$

$$\frac{\mathrm{d}\mathbf{M}}{\mathrm{d}t} = \frac{\mathrm{d}}{\mathrm{d}t}\sum_i \boldsymbol{\mu}_i = \sum_i \frac{\mathrm{d}\boldsymbol{\mu}_i}{\mathrm{d}t} = \sum_i \gamma(\boldsymbol{\mu}_i \times \mathbf{B}) = \gamma\left(\sum_i \boldsymbol{\mu}_i\right) \times \mathbf{B} = \gamma(\mathbf{M} \times \mathbf{B})$$

Problem 3: on page 6

Simply write the cross product in determinant form, then expand the determinant in minors of the first row.

$$\begin{aligned}\frac{\mathrm{d}\mathbf{M}}{\mathrm{d}t} = \gamma(\mathbf{M} \times \mathbf{B}) &= \gamma \begin{vmatrix} \mathbf{i} & \mathbf{j} & \mathbf{k} \\ M_x & M_y & M_z \\ B_x & B_y & B_z \end{vmatrix} \\ &= \gamma\left(\begin{vmatrix} M_y & M_z \\ B_y & B_z \end{vmatrix}\mathbf{i} - \begin{vmatrix} M_x & M_z \\ B_x & B_z \end{vmatrix}\mathbf{j} + \begin{vmatrix} M_x & M_y \\ B_x & B_y \end{vmatrix}\mathbf{k}\right) \\ &= \gamma\left[\left(M_y B_z - M_z B_y\right)\mathbf{i} - \left(M_x B_z - M_z B_x\right)\mathbf{j} + \left(M_x B_y - M_y B_x\right)\mathbf{k}\right]\end{aligned}$$

which leads directly to Equation (7).

Problem 4: on page 8

Simply eliminate all terms involving B_x or B_y from Equation (7) to yield Equation (8). Now differentiate Equation (9) with respect to time to yield:

$$\begin{aligned}\frac{\mathrm{d}\mathbf{M}}{\mathrm{d}t} &= -M_o\omega_o \sin(\omega_o t)\mathbf{i} - M_o\omega_o \cos(\omega_o t)\mathbf{j} \\ &= -M_o\gamma B_o \sin(\omega_o t)\mathbf{i} - M_o\gamma B_o \cos(\omega_o t)\mathbf{j} \\ &= \gamma M_y B_o \mathbf{i} - \gamma M_x B_o \mathbf{j} \\ &= \gamma M_y B_z \mathbf{i} - \gamma M_x B_z \mathbf{j}\end{aligned}$$

The last step uses the fact that $B_o = B_z$.

Problem 5: on page 10

First one must invert the transformation Equation (10) to find

$$\begin{bmatrix} x \\ y \\ z \end{bmatrix} = \begin{bmatrix} \cos(\omega t) & -\sin(\omega t) & 0 \\ \sin(\omega t) & \cos(\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x' \\ y' \\ z' \end{bmatrix}$$

Applying this transformation to **M** one obtains

$$\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} \cos(\omega t) & -\sin(\omega t) & 0 \\ \sin(\omega t) & \cos(\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix}$$

and differentiating yields

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} \cos(\omega t) & -\sin(\omega t) & 0 \\ \sin(\omega t) & \cos(\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix} \frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix} + \omega \begin{bmatrix} -\sin(\omega t) & -\cos(\omega t) & 0 \\ \cos(\omega t) & -\sin(\omega t) & 0 \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix}$$

Now, writing the differential Equation (7) in matrix form, yields

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \gamma \begin{bmatrix} 0 & B_z & -B_y \\ -B_z & 0 & B_x \\ B_y & -B_x & 0 \end{bmatrix} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix}$$

and substituting the transformed magnetization gives

$$\begin{bmatrix} \cos(\omega t) & -\sin(\omega t) & 0 \\ \sin(\omega t) & \cos(\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix} \frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix} + \omega \begin{bmatrix} -\sin(\omega t) & -\cos(\omega t) & 0 \\ \cos(\omega t) & -\sin(\omega t) & 0 \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix}$$
$$= \gamma \begin{bmatrix} 0 & B_z & -B_y \\ -B_z & 0 & B_x \\ B_y & -B_x & 0 \end{bmatrix} \begin{bmatrix} \cos(\omega t) & -\sin(\omega t) & 0 \\ \sin(\omega t) & \cos(\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix}$$

which, multiplying from the left by the inverse transformation matrix, simplifies to

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix} + \omega \begin{bmatrix} \cos(\omega t) & \sin(\omega t) & 0 \\ -\sin(\omega t) & \cos(\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} -\sin(\omega t) & -\cos(\omega t) & 0 \\ \cos(\omega t) & -\sin(\omega t) & 0 \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix}$$

$$= \gamma \begin{bmatrix} \cos(\omega t) & \sin(\omega t) & 0 \\ -\sin(\omega t) & \cos(\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 0 & B_z & -B_y \\ -B_z & 0 & B_x \\ B_y & -B_x & 0 \end{bmatrix} \begin{bmatrix} \cos(\omega t) & -\sin(\omega t) & 0 \\ \sin(\omega t) & \cos(\omega t) & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix} + \omega \begin{bmatrix} 0 & -1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix} = \gamma \begin{bmatrix} 0 & B'_z & -B'_y \\ -B'_z & 0 & B'_x \\ B'_y & -B'_x & 0 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix} = \gamma \begin{bmatrix} 0 & B'_z + \dfrac{\omega}{\gamma} & -B'_y \\ -\left(B'_z + \dfrac{\omega}{\gamma}\right) & 0 & B'_x \\ B'_y & -B'_x & 0 \end{bmatrix} \begin{bmatrix} M'_x \\ M'_y \\ M'_z \end{bmatrix}$$

If ω is taken to be intrinsically negative, this is the desired result. This is reasonable since our original equation says that the magnetization precesses in a negative sense when γ is positive. (See Figure 2.)

Problem 6: on page 12

Equation (13) represents a field rotating in a positive sense. If one adds this to a similar equation with ω replaced by -ω, the result is:

$$\begin{aligned} \mathbf{B}_1^+ + \mathbf{B}_1^- &= B_1\left[\cos\left(\omega_{rf}t\right)\mathbf{i} + \sin\left(\omega_{rf}t\right)\mathbf{j}\right] + B_1\left[\cos\left(-\omega_{rf}t\right)\mathbf{i} + \sin\left(-\omega_{rf}t\right)\mathbf{j}\right] \\ &= B_1\left[\cos\left(\omega_{rf}t\right)\mathbf{i} + \sin\left(\omega_{rf}t\right)\mathbf{j}\right] + B_1\left[\cos\left(\omega_{rf}t\right)\mathbf{i} - \sin\left(\omega_{rf}t\right)\mathbf{j}\right] \\ &= 2B_1\cos\left(\omega_{rf}t\right)\mathbf{i} \end{aligned}$$

Thus, there remains only the oscillatory x component of the field.

Problem 7: on page 12

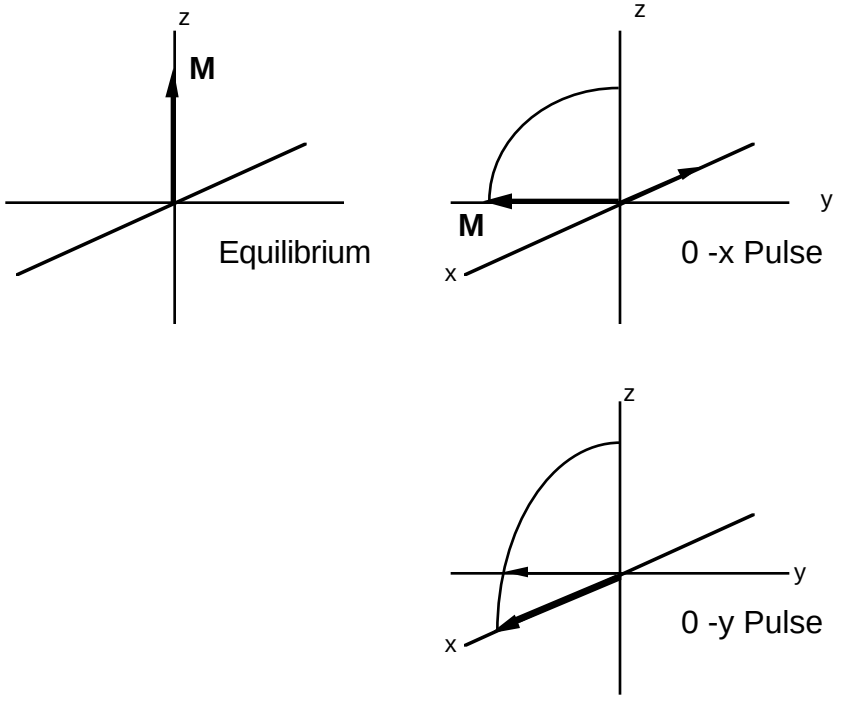
It has been shown previously that, ignoring $\mathbf{B}_1$, the above conditions lead to $\mathbf{B}_{\mathbf{eff}} = 0$. Hence, one can transform $\mathbf{B}_1$ into the rotating frame to obtain:

$$\begin{aligned}\mathbf{B}_1 &= B_1\begin{bmatrix}\cos(\omega t) & \sin(\omega t)\\ -\sin(\omega t) & \cos(\omega t)\end{bmatrix}\begin{bmatrix}\cos\left(\omega_{rf}t\right)\\ \sin\left(\omega_{rf}t\right)\end{bmatrix}\\ &= B_1\begin{bmatrix}\cos(\omega t)\cos\left(\omega_{rf}t\right)+\sin(\omega t)\sin\left(\omega_{rf}t\right)\\ -\sin(\omega t)\cos\left(\omega_{rf}t\right)+\cos(\omega t)\sin\left(\omega_{rf}t\right)\end{bmatrix}\\ &= B_1\begin{bmatrix}\cos\left[\left(\omega+\omega_{rf}\right)t\right]\\ \sin\left[\left(\omega+\omega_{rf}\right)t\right]\end{bmatrix}\end{aligned}$$

and, if $-\omega = \omega_{rf}$ then

$$\mathbf{B}_1 = B_1\begin{bmatrix}1\\ 0\end{bmatrix}$$

Problem 8: on page 13



Problem 9: on page 13

As shown in Figure 7, the magnetization rotates about $\mathbf{B}_{eff}$ not $\mathbf{B}_1$. Suppose the angle between $\mathbf{B}_{eff}$ and $\mathbf{B}_1$ is $\theta = \tan^{-1}\left[\left(B_0 - \omega_{rf}/\gamma\right)/B_1\right]$, then the closest approach of $\mathbf{M}$ to the negative z-axis is 2θ because $\mathbf{B}_{eff}$ must bisect the vertex angle of the cone traced out by $\mathbf{M}$ as it precesses.

Problem 10: on page 13

The time required will be shorter because the precession is controlled by $\mathbf{B}_{eff}$, and $\mathbf{B}_{eff}$, is larger than $\mathbf{B}_1$ so the precession is faster. The phase will be different because the cone does not intersect the *xy* plane exactly on the *x*-axis. The strength of the $\mathbf{B}_1$ field is at most a few tens of kilohertz while the carbon-13 is many megahertz off resonance. Thus, $\mathbf{B}_{eff}$ is essentially colinear with the positive *z*-axis and cannot perturb the spins. The vertex angle of the cone is essentially zero.

Problem 11: on page 15

Differentiating the proposed solution gives:

$$\frac{\mathrm{d}M}{\mathrm{d}t} = -\alpha A \mathrm{e}^{-\alpha t} = -\alpha(M - C)$$

Comparing this form with the given equations, α may be identified with $1/T_2$ in the firs two equations and with $1/T_1$ in the third. The constant, C, is clearly zero in the first two cases and $-M_o$ in the third. The constant, A, must be identified with the usual constant of integration and can be evaluated by noting that, at $t = 0$, $A = M - C$. Hence, one cannot know precisely what A is until the value of $\mathbf{M}$ at $t = 0$ is specified.

Problem 12: on page 15

In this case (see Figure 5) one can define the effective field to be

$$\Delta B = \frac{\omega_o - \omega}{\gamma}$$

Substituting this equation into Equation (15) yields:

$$\frac{\mathrm{d}M_x}{\mathrm{d}t} = \gamma M_y \Delta B - \frac{M_x}{T_2}$$
$$\frac{\mathrm{d}M_y}{\mathrm{d}t} = -\gamma M_x \Delta B - \frac{M_y}{T_2}$$
$$\frac{\mathrm{d}M_z}{\mathrm{d}t} = -\frac{M_z - M_0}{T_1}$$

Problem 13: on page 21

For convenience the matrix form of Equation (18) will be used.

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} -\frac{1}{T_2} & \gamma\Delta B & 0 \\ -\gamma\Delta B & -\frac{1}{T_2} & \gamma B_1 \\ 0 & -\gamma B_1 & -\frac{1}{T_1} \end{bmatrix}\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ \frac{M_0}{T_1} \end{bmatrix}$$

The steady state solution is formed by setting the derivatives to zero and solving the resulting coupled linear equations discarding all terms higher than first order in B_1.

$$\begin{bmatrix} -\frac{1}{T_2} & \gamma\Delta B & 0 \\ -\gamma\Delta B & -\frac{1}{T_2} & \gamma B_1 \\ 0 & -\gamma B_1 & -\frac{1}{T_1} \end{bmatrix}\begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ -\frac{M_0}{T_1} \end{bmatrix}$$

Evaluating the determinant of coefficients produces the denominator of the solutions.

$$\begin{vmatrix} -\frac{1}{T_2} & \gamma\Delta B & 0 \\ -\gamma\Delta B & -\frac{1}{T_2} & \gamma B_1 \\ 0 & -\gamma B_1 & -\frac{1}{T_1} \end{vmatrix} = -\frac{1}{T_1 T_2^{\,2}} - \frac{(\gamma\Delta B)^2}{T_1} = -\frac{1 + (\omega - \omega_o)^2 T_2^{\,2}}{T_1 T_2^{\,2}}$$

Substituting the right hand side into the determinant for the numerator in the usual way gives

$$M_x = -\frac{T_1 T_2^{\,2}}{1+(\omega-\omega_o)^2 T_2^{\,2}} \begin{vmatrix} 0 & \omega-\omega_o & 0 \\ 0 & -\frac{1}{T_2} & \gamma B_1 \\ -\frac{M_0}{T_1} & -\gamma B_1 & -\frac{1}{T_1} \end{vmatrix}$$

$$= \frac{M_o T_2^{\,2}(\omega-\omega_o)\gamma B_1}{1+(\omega-\omega_o)^2 T_2^{\,2}} = \left(\frac{M_o}{B_o}\right)(\gamma B_o) T_2 \frac{(\omega-\omega_o)T_2}{1+(\omega-\omega_o)^2 T_2^{\,2}} B_1$$

$$= \chi_o \omega_o T_2 \frac{(\omega-\omega_o)T_2}{1+(\omega-\omega_o)^2 T_2^{\,2}} B_1$$

$$M_y = -\frac{T_1 T_2^{\,2}}{1+(\omega-\omega_o)^2 T_2^{\,2}} \begin{vmatrix} -\frac{1}{T_2} & 0 & 0 \\ -\gamma\Delta B & 0 & \gamma B_1 \\ 0 & -\frac{M_0}{T_1} & -\frac{1}{T_1} \end{vmatrix}$$

$$= \frac{T_2 M_o \gamma B_1}{1+(\omega-\omega_o)^2 T_2^{\,2}} = \left(\frac{M_o}{B_o}\right)(\gamma B_o) T_2 \frac{1}{1+(\omega-\omega_o)^2 T_2^{\,2}} B_1$$

$$= \chi_o \omega_o T_2 \frac{1}{1+(\omega-\omega_o)^2 T_2^{\,2}} B_1$$

$$M_z = -\frac{T_1 T_2^{\,2}}{1+(\omega-\omega_o)^2 T_2^{\,2}} \begin{vmatrix} -\frac{1}{T_2} & \omega-\omega_o & 0 \\ -(\omega-\omega_o) & -\frac{1}{T_2} & 0 \\ 0 & -\gamma B_1 & -\frac{M_o}{T_1} \end{vmatrix}$$

$$= \frac{T_1 T_2^{\,2}}{1+(\omega-\omega_o)^2 T_2^{\,2}} M_o \left(\frac{1}{T_1 T_2^{\,2}} + \frac{(\omega-\omega_o)^2}{T_1}\right) = M_o$$

Problem 14: on page 22

First Equation (20) will be recast in terms of Hertz.

$$F_x(\nu) = AT_2 \frac{2\pi(\nu - \nu_o)T_2}{1 + 4\pi^2(\nu - \nu_o)^2 T_2^{\,2}} B_1$$

$$F_y(\nu) = AT_2 \frac{1}{1 + 4\pi(\nu - \nu_o)^2 T_2^{\,2}} B_1$$

The graphs below show the curves for two different values of T_2. Since the equations are strictly linear in $\mathbf{B}_1$, it will only scale the curves vertically.

The fwhm point is found as follows:

$$F_y(0) = AT_2 B_1$$

$$F_y(\nu_{1/2}) = \frac{AT_2 B_1}{2} = AT_2 \frac{1}{1 + 4\pi^2(\nu - \nu_o)^2 T_2^{\,2}} B_1$$

$$\frac{1}{2} = \frac{1}{1 + 4\pi^2(\nu - \nu_o)^2 T_2^{\,2}}$$

$$(\nu - \nu_o)^2 = \frac{1}{4\pi^2 T_2^{\,2}}$$

$$\nu - \nu_o = \pm \frac{1}{2\pi T_2}$$

$$fwhm = \frac{1}{\pi T_2}$$

To find the 1% point one simply replaces 2 by 100 in the above equation to give

$$F_y(0) = AT_2 B_1$$

$$F_y(\nu_{0.01}) = \frac{AT_2 B_1}{100} = AT_2 \frac{1}{1 + 4\pi^2(\nu - \nu_o)^2 T_2^{\,2}} B_1$$

$$\frac{1}{100} = \frac{1}{1 + 4\pi^2(\nu - \nu_o)^2 T_2^{\,2}}$$

$$(\nu - \nu_o)^2 = \frac{99}{4\pi^2 T_2^{\,2}}$$

$$\nu - \nu_o = \pm \frac{\sqrt{99}}{2\pi T_2} = \pm \frac{\sqrt{99}}{2} \left(\frac{1}{\pi T_2} \right)$$

So the 1% point is about five line-widths off resonance. Similarly for the dispersion mode

$$F_y(0) = AT_2B_1$$

$$F_x(\nu_{0.01}) = \frac{AT_2B_1}{100} = AT_2\frac{2\pi(\nu-\nu_o)T_2}{1+4\pi^2(\nu-\nu_o)^2T_2^2}B_1$$

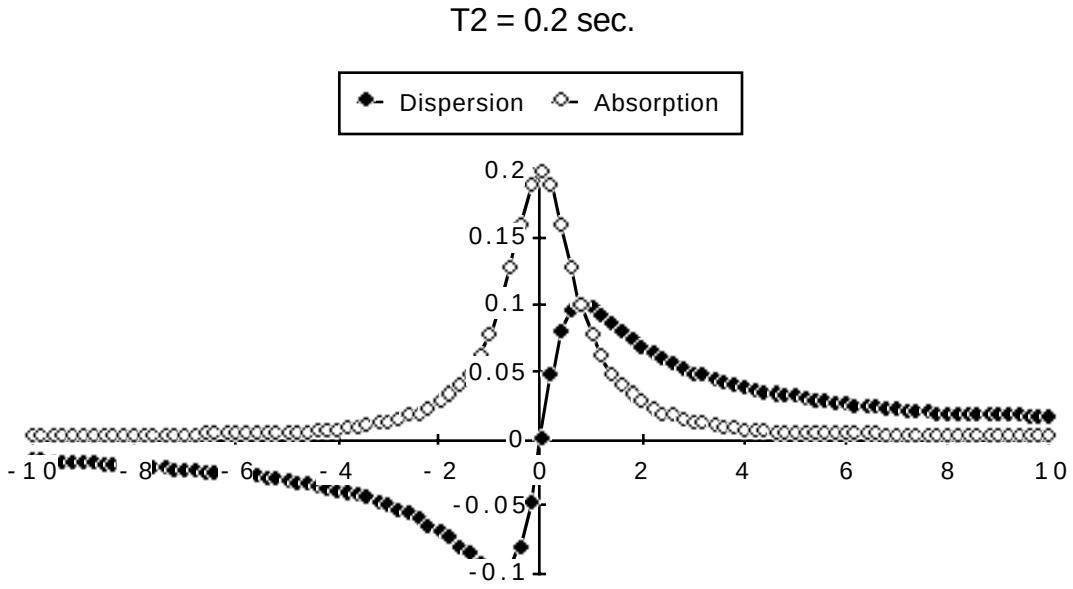
$$\frac{1}{100} = \frac{2\pi(\nu-\nu_o)T_2}{1+4\pi^2(\nu-\nu_o)^2T_2^2}$$

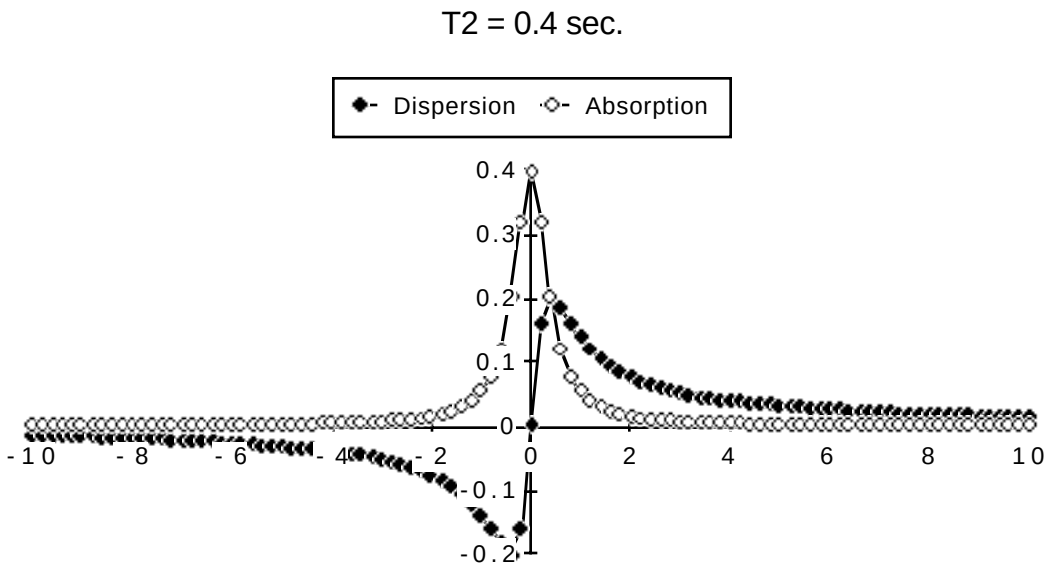
Assume that one is negligible compared to the off-resonance term to give

$$\frac{1}{100} = \frac{2\pi(\nu-\nu_o)T_2}{4\pi^2(\nu-\nu_o)^2T_2^2} = \frac{1}{2\pi(\nu-\nu_o)T_2}$$

$$\nu-\nu_o = 50\left(\frac{1}{\pi T_2}\right)$$

This says that the 1% point is ten times further off resonance for the dispersion mode than for the absorption mode.





Problem 15: on page 24

From Equation (21) the equation for the complex FID is

$$M_x = M_0 e^{-(t/T_2)} \sin\left[(\omega_0 - \omega)t\right]$$
$$M_y = M_0 e^{-(t/T_2)} \cos\left[(\omega_0 - \omega)t\right]$$

If the sign of ω - ω_0 is changed, the sign of M_x will change. This indicates that the magnetization is precessing in the opposite sense in the rotating frame.

Problem 16: on page 27

The methyl protons of tert-butyl alcohol are all equivalent, so the ratio of the methyl to the hydroxyl protons would be nine to one. The largest number expressible in three bits is eight and in four bits, sixteen; so four bits would be enough for the magnitude of the number, but one more bit is needed to store the sign, so a total of five bits would be needed. However, if a tert-butyl group is used as a bulky substituent in a molecule, other single proton resonances might be split into multiplets reducing the size of each resonance so that more bits would be needed.

Pure water is 55 M, so it would be 110 M in protons, but 10% of the hydrogen is deuterium so the solution is about 100 M in protons. The ratio of the two resonances would

then be about 20,000:1. Using a 15 bit ADC (the size used on the VXR-500) would permit storage of $\pm 2^{14} = \pm 16{,}384$; this is not enough; one would need 16 bits. This illustrates why it is necessary to use solvent suppression techniques for obtaining spectra of dilute biological samples in water solution. Note also that a 12 bit ADC (such as used on the XL-300) would be completely inadequate for this kind of work.

Problem 17: on page 28

The data points representing the two lines would have to be separated by at least one data point of lower intensity than either of them, so the two lines would have to be at least 2/A apart. The only values that could be found for a coupling constant would be 2/A, 3/A, 4/A,...; hence, a reasonable error limit would be $\pm 1/2A$. Interpolation schemes can do somewhat better than this if the signal to noise is good.

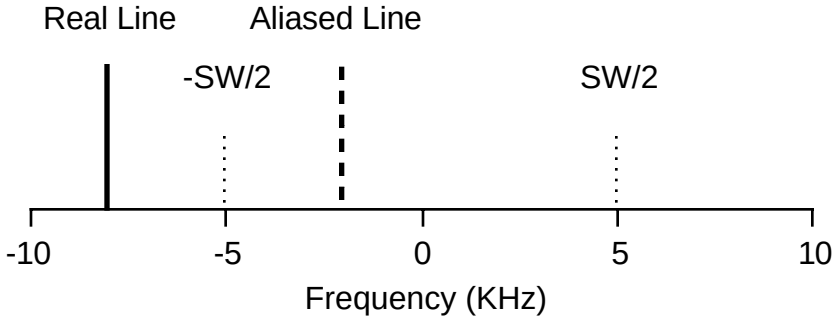
Problem 18: on page 29

Suppose your spectrometer has 128 K bytes available for storing the FID; then, if two bytes (16 bits) are used to store a real number, four bytes are needed for each complex sample of the FID to give a value of 32 K or 32,768 for N_{max}. On a 400 MHz machine the nominal spectral widths would be $10 \text{ ppm} \times 400 \text{ Hz/ppm} = 4000 \text{ Hz}$ for protons and $200 \text{ ppm} \times 100 \text{ Hz/ppm} = 20{,}000 \text{ Hz}$ for carbon Using these spectral widths gives 0.12 Hz for protons and 0.61 Hz for carbon. One could use a spectral width of $32{,}768 \times 0.2 = 6554$ Hz for any experiment while maintaining 0.2 Hz resolution. Note that, if double precision is used (8 bytes per complex point), all of these values must be reduced by a factor of two.

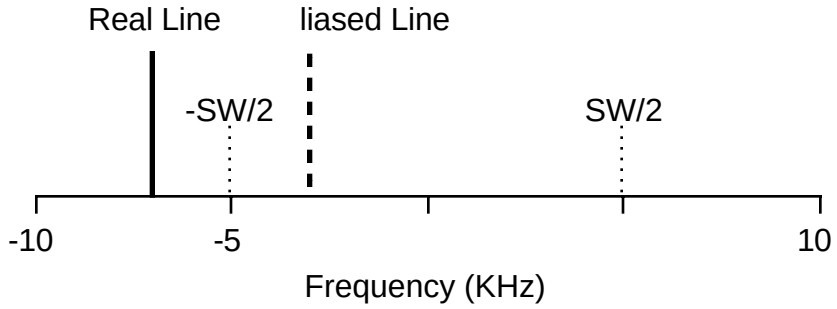
Problem 19: on page 29

Changing the frequency of the transmitter or the spectral width does not change the Larmor frequency of a resonance; hence, if a line moves in the spectrum in a way that is inconsistent with this fact, it must not be a real line. Consider the following examples:

Single Sideband Detection

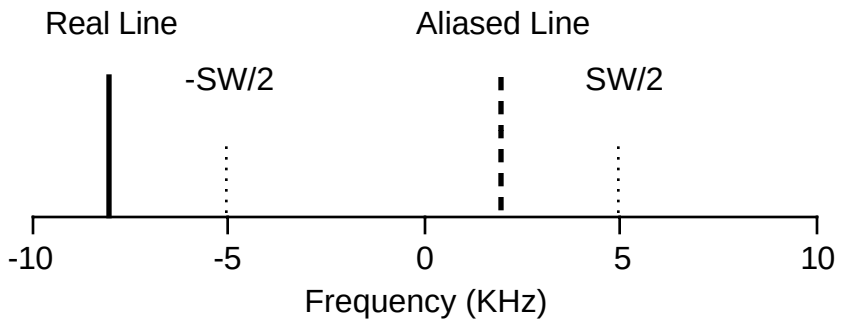


Transmitter Frequency Decreased by 1 KHz

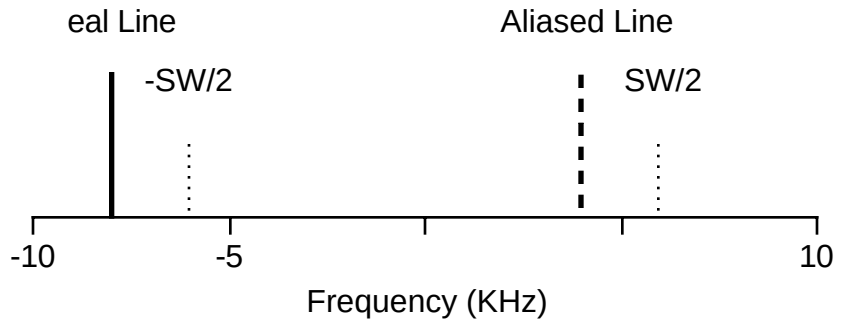


The line should have appeared at 1 KHz; instead it appeared at 3 KHz.

Quadrature Detection



Spectral Width Increased by 2 KHz



The position of the line should not have changed; instead it moved from 2 KHz to 4 KHz.

Many other examples can be analyzed.

Problem 20: on page 31

In order to improve the signal to noise by a factor of 5, one must take 25 times as many transients. Since 12 hr. have already been invested, it would take $25 \times 12 = 300$ hr. = 12.5 days to complete the job.

Problem 21: on page 35

The ratio of the time required using the direct calculation to that using the FFT would be

$$\frac{N^2}{N \log_2 N}$$

Substituting $1024 = 2^{10}$ yields 102.4, or it would take more than 100 times longer to do the calculation directly. For 32 K the result is 2184.5 or more than 2000 times longer. Thus, if it were necessary to do line broadening by the direct computation of the convolution, something that takes 10 sec. would take over 6 hr.

Problem 22: on page 38

Equation (21) gives the form we have used previously.

$$M_x = M_o \, \mathrm{e}^{-\frac{t}{T_2}} \sin(\omega t)$$
$$M_y = M_o \, \mathrm{e}^{-\frac{t}{T_2}} \cos(\omega t)$$

Using the familiar identity

$$\mathrm{e}^{-ix} = \cos(x) - i\sin(x)$$

yields

$$M_y + iM_x = M_o \, \mathrm{e}^{-\frac{t}{T_2}} (\cos(\omega t) - i\sin(\omega t))$$
$$= M_o \, \mathrm{e}^{-\frac{t}{T_2}} \mathrm{e}^{-i\omega t} = M_o \, \mathrm{e}^{\left(-i\omega t - \frac{t}{T_2}\right)}$$

Multiplying Equation (39) and Equation (40) together gives

$$f(t) f_c(t) = M_o \, \mathrm{e}^{\left(-i\omega t - \frac{t}{T_2}\right)} \mathrm{e}^{\left(-\frac{t}{T_{LB}}\right)}$$
$$= M_o \, \mathrm{e}^{\left(-i\omega t - t\left(\frac{1}{T_2} + \frac{1}{T_{LB}}\right)\right)}$$

Thus, the decay rate associated with the convolved FID is the sum of the two individual decay rates. We know that the full width at half maximum of a Lorentzian is

$$\frac{1}{\pi T_2}$$

If $/ T_2$ is replaced by $/ T_2 + 1 / T_{LB}$, the desired result follows immediately.

Problem 23: on page 39

Taking the reciprocal of the exponential yields

$$f_c(t) = \exp\left(+\frac{t}{T_{LB}}\right) = \frac{1}{\exp\left(-\frac{t}{T_{LB}}\right)}$$

Thus, multiplying by the positive exponential is the same as dividing by the negative exponential which corresponds to a deconvolution with a Lorentzian resulting in a narrowing of the lines in the spectrum.

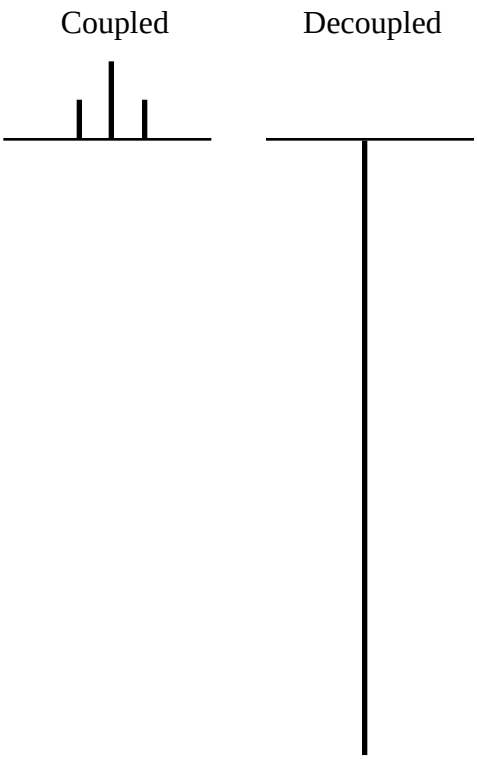
Problem 24: on page 43

We write the equation for the carbon magnetization from Equation (44) then impose steady state and saturation of the protons

$$\begin{aligned}
\frac{\mathrm{d}M_z^C}{\mathrm{d}t} &= -\rho_C\left(M_z^C(t) - M_z^C(\infty)\right) - \sigma\left(M_z^H(t) - M_z^H(\infty)\right) = 0 \\
\rho_C M_z^C &- \rho_C M_z^C(\infty) - \sigma M_z^H(\infty) = 0 \\
M_z^C &= M_z^C(\infty) + \frac{\sigma}{\rho_C} M_z^H(\infty) \\
&= M_z^C(\infty) + \frac{\sigma}{\rho_C}\left(\frac{\gamma_H}{\gamma_C} M_z^C(\infty)\right) \\
&= M_z^C(\infty)\left(1 + \frac{\gamma_H \sigma}{\gamma_C \rho_C}\right) \\
&= \left(1 + \eta_{C\{H\}}\right) M_z^C(\infty)
\end{aligned}$$

Problem 25: on page 43

The coupled spectrum is a triplet with 1:2:1 intensity ratios due to the coupling to the amide protons. The ratio of the γ's is about -10; at constant field the ratio of the γ's is the same as the ratio of the Larmor frequencies. Thus, the value of η is -5 and the value of the NOE is -4. So, the intensity of the decoupled spectrum is four times greater than the sum of the intensities of the triplet lines and will be negative as shown in the following figure.

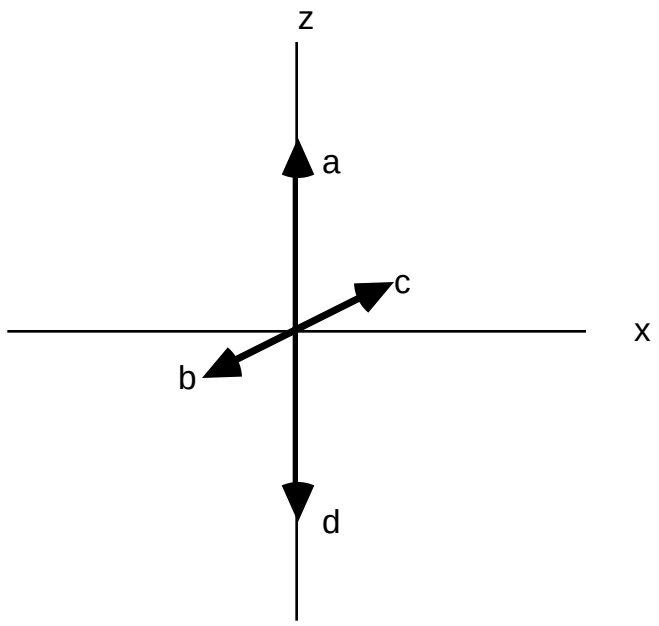


Problem 26: on page 43

In this case the gyromagnetic ratios cancel from the equation, and one is left with an NOE of 1.5.

Problem 27: on page 45

Consider the projection of the magnetization on the xz-plane. Beginning at thermal equilibrium at point a, an off resonance 90(x) pulse will take the magnetization to point b. The 180(y) pulse will then take the magnetization approximately to point c. The final 90(x) pulse then takes the magnetization to point d which is a far more accurate 180(x) pulse than would otherwise have resulted.

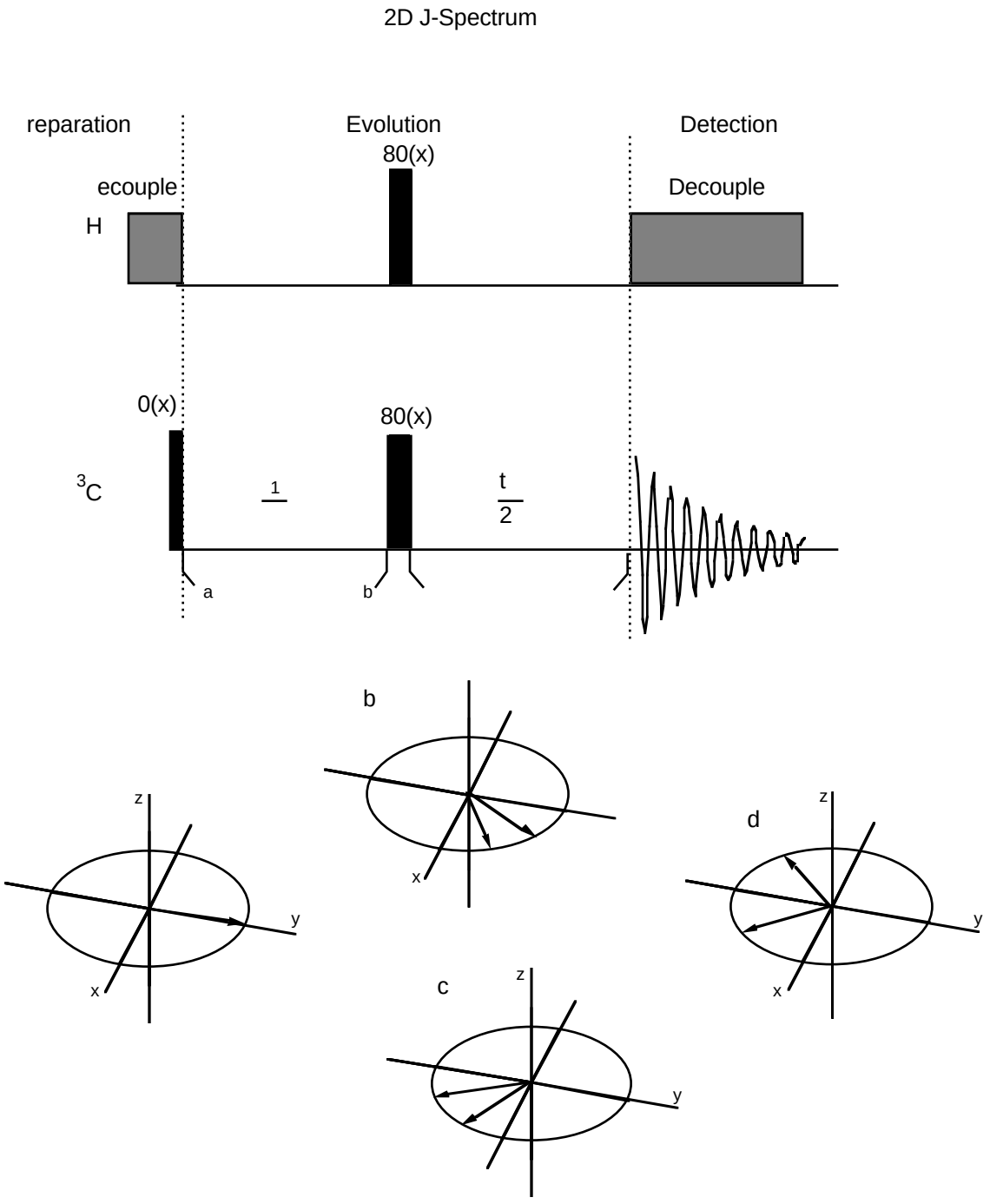


Problem 28: on page 47

The magnetization will refocus alternately along the positive and negative y-axis, so the lines in the transformed spectrum will be alternately positive and negative in amplitude. However, minor errors in the 180 degree pulse will leave the magnetization much further from the y-axis, where it ideally ought to be, than would be the case with the phase shifted pulse sequence.

Problem 29: on page 68

The following figure shows the modified pulse sequence. During the first half of t_1 both chemical shift and scalar coupling evolve. The simultaneous 180 degree pulses rotate the magnetization about the *x*-axis as shown, but the two vectors are interchanged as we have seen before, so that the chemical shift is refocussed along the negative *y*-axis while the scalar coupling continues to evolve. Since the scalar coupling continues to evolve during the entirety of t_1, the scalar couplings develop their full value in contrast to the gated decoupler method where they evolve during only half of t_1 and develop only half of their actual value.



Problem 30: on page 68

The only difference between this experiment and the heteronuclear case is that it is impossible to do broadband decoupling during acquisition. Thus, scalar coupling is present in both F_1 and F_2, but chemical shifts are present only in F_2. The vector diagrams shown

for the previous problem are completely relevant. The chemical shift is refocussed, but the scalar coupling is not. Since the 180 degree pulse is nonselective, it inverts both doublets of the proton AX spin system just as the simultaneous pulses on protons and carbon did in the heteronuclear case.